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Short communication

Sporadic myopathy, myoclonus, leukoencephalopathy, neurosensory deafness, hypertrophic cardiomyopathy and insulin resistance associated with the mitochondrial 8306 T>C *MTTK* mutation

Elena Cardaioli [a,1], Edoardo Malfatti [a,b,1], Carla Battisti [a], Paola Da Pozzo [a], Anna Rubegni [a], Gian Nicola Gallus [a], Alessandro Malandrini [a], Antonio Federico [a,*]

[a] Department of Neurological, Neurosurgical and Behavioural Sciences, University of Siena, Siena, Italy
[b] Unité de Morphologie Neuromusculaire Institut de Myologie, GH Pitié-Salpêtrière, Paris, France

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ABSTRACT

We report a new T8306C transition in the D-stem of the *MTTK* gene of a 67-year-old man who manifested severe adult onset myopathy, myoclonus, leukoencephalopathy, neurosensory hypoacusis, hypertrophic cardiomyopathy and insulin resistance. No other family member was affected, suggesting that our patient was a sporadic case. The T8306C mutation was heteroplasmic in several tissues of the proband, while it was absent from his asymptomatic siblings. Single fibre analysis confirmed the segregation of higher mutational load in cytochrome c oxidase-deficient fibres. The mutation T8306C is predicted to disrupt a highly conserved base pair and was not found in more than 120 controls. This finding broadens the phenotypic and molecular spectrum of mitochondrial $tRNA^{Lys}$ associated disorders.

1. Introduction

Mitochondrial encephalomyopathies are a heterogeneous group of diseases often associated with multisystem involvement [1]. The most severely affected organs are those with high oxidative metabolism, such as the brain, skeletal and heart muscles, kidneys and endocrine glands [2]. However, virtually any organ or tissue in the body may be affected and the respective disorders may be multisystemic (mitochondrial encephalomyopathies) or confined to a single tissue [3]. More than 200 mtDNA point mutations have so far been associated with a wide spectrum of clinical manifestations; those involving tRNA appear to be the most common (http://www.mitomap.org). Most pathogenic point mutations that alter tRNA genes are heteroplasmic and usually associated with significant mitochondrial morphological alterations, ragged red fibres (RRFs), cytochrome c oxidase (COX) defective fibres and combined respiratory chain defects in muscle [4]. This is presumably due to the essential role of tRNAs in the synthesis of proteins involved in energy metabolism [5]. Inheritance is maternal in most cases, though some sporadic cases have been described.

Here we report a new pathogenic heteroplasmic mutation in the D-stem of the *MTTK* gene of a 67-year-old man with severe myopathy, diaphragm myoclonus, leukoencephalopathy, neurosensory hypoacusis, hypertrophic cardiomyopathy and insulin resistance.

2. Case report

A 67-year-old Italian man with progressive difficulty in walking and raising the arms, and dysphagia, was referred to our unit. There was no family history of muscle disease and no parental consanguinity. Muscle symptoms occurred around the age of 30 years when the subject began to have difficulty climbing stairs, rising from the floor or a chair, and walking for long distances. He subsequently developed upper limb weakness and difficulty raising the arms. These symptoms took a progressive course. Unaided walking was maintained. At the age of 64 years, cardiac arrhythmia developed, followed by rhythmic contractions in the abdominal region, defined as diaphragm myoclonus, at 66 years.

Examination showed paraspinal muscle atrophy with loss of normal spinal curvature, lumbar kyphosis, thoracic lordosis and neck hyperextension, as well as bilateral symmetrical atrophy of deltoid and sternocleidomastoid muscles and bilateral foot drop. Neurological

* Corresponding author at: Department of Neurological, Neurosurgical and Behavioural Sciences, University of Siena, Viale Bracci 2, 53100 Siena, Italy. Tel.: +39 0577 585763 60; fax: +39 0577 40327.
E-mail address: federico@unisi.it (A. Federico).
[1] These authors contributed equally.

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Table 1
Respiratory chain activities in the muscle, showing decreased activity of complexes I, III, and IV. Complex II activity is slightly reduced. Decreased activities are in bold.

	NADH-CoQ_1 red (complex I)	Succ-CoQ_1 red (complex II)	DBH_2 Cyt c red (complex III)	COX (complex IV)	ATPase (complex V)	CS
Patient	**6**	**12**	**70**	**76**	253	113
Control mean (n. 35)	15.3 ± 5.8	22.5 ± 6.5	127.8 ± 41	154 ± 51	200 ± 70	174 ± 61

The activities are expressed as nmol/min mg protein and normalised to that of citrate synthase (CS). Control values ± s.d.

examination revealed very mild lateral gaze-evoked nystagmus and absence of osteotendinous reflexes (ROTs). The patient also reported dysphagia of liquids and solids. Manual muscle testing showed proximal upper limb weakness involving deltoids and sternocleidomastoids (MRC grade 2) and lower limb weakness especially of the psoas (MRC grade 2) and tibialis anterior (MRC grade 2). Axial weakness involved the neck flexors (MRC grade 2/5) and caused difficulty in rising from supine to sitting position. Serum creatine kinase was mildly elevated (250 IU/l normal range <170 IU/l). Venous lactate and pyruvate were within normal limits. Blood glucose showed a profile compatible with insulin resistance. Electromyography detected a diffuse chronic myogenic pattern. Muscle CT scan showed bilateral symmetrical atrophy/fatty replacement of the posterior paravertebral and iliopsoas muscles. Lung function tests revealed severe restrictive and obstructive hypercapnic respiratory failure with forced vital capacity reduced to 45%. Polysomnography detected nocturnal hypoventilation and frequent short periods of apnea. Non invasive nocturnal ventilation was implemented during and after hospitalisation. Echocardiography showed mild left ventricular hypertrophy.

Audiometry showed neurosensory hypoacusis. EEG indicated diffuse slow activity. Brain CT scan revealed bilateral calcification of the basal nuclei. Brain MRI showed bilateral hyperintense T2 signal in white matter.

Biopsy of the left quadriceps/deltoid muscle at age 67 years and Gomori trichrome stain revealed 5% RRFs (Fig. 2B). Histochemical staining revealed 45% COX-negative fibres (Fig. 2B). Some hypotrophic fibres contained rimmed vacuoles. Muscle biochemical analysis [6] revealed deficiency in respiratory chain complexes I, III and IV (Table 1). Complex II activity was also slightly reduced.

2.1. Molecular genetic study

Total DNA was isolated from the skeletal muscle, blood, hair roots and urinary and buccal epithelial cells of the proband and from the blood, hair and urinary and buccal epithelium of the brother and sister of the patient, using the QIAmp DNA Mini Kit (Qiagen). Screening for large-scale rearrangements of mtDNA, performed on muscle by long PCR, was negative. The whole mitochondrial genome, amplified by PCR from muscle DNA and investigated by sequence analysis, revealed a T>C transition at position 8306 (Fig. 1A) that disrupted base pairing in the dihydrouridine stem (DHU stem) of $tRNA^{Lys}$ (Fig. 2). This Watson-Crick base pairing is highly conserved throughout evolution (Fig. 1B). The mutation was confirmed by unlabelled PCR-Restriction Fragment Length Polymorphisms (RFLP) analysis. The DNA was amplified using the sense primer 8201–8222, 5′-TTCATG

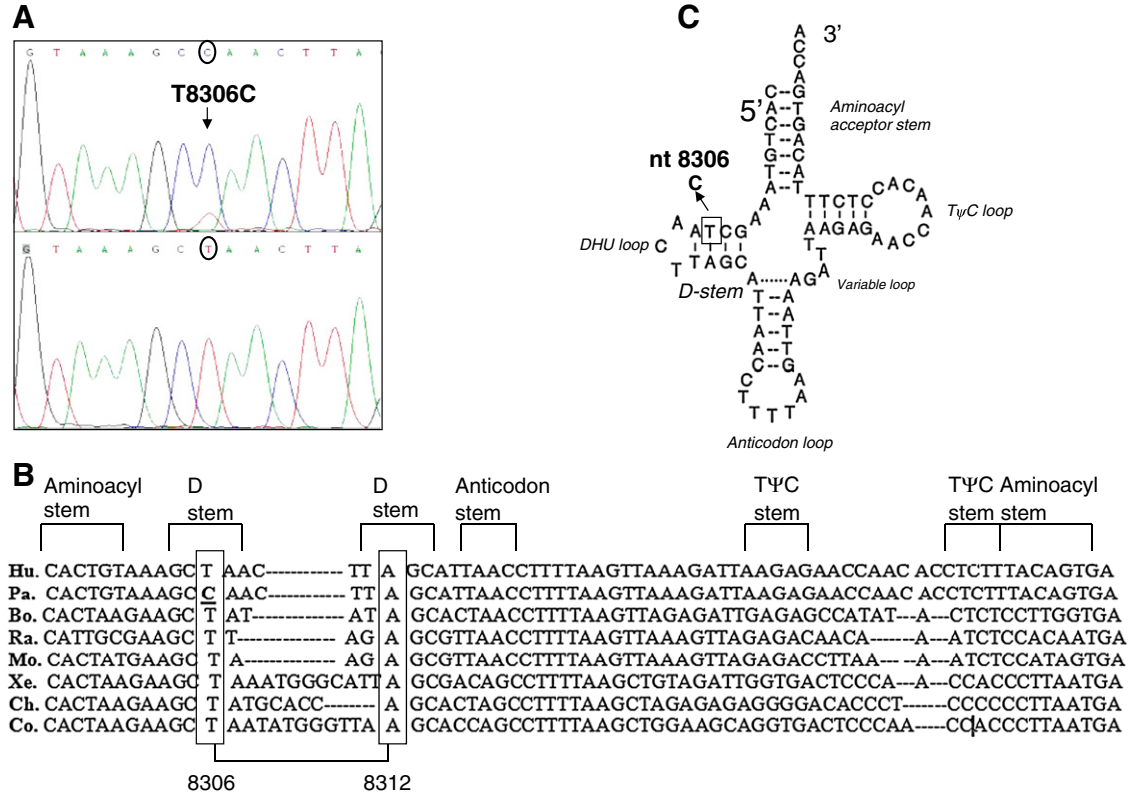

Fig. 1. (A) Identification of the T8306C mutation by direct DNA sequencing of the *MTTK* gene. Electropherograms of nucleotide positions 8298–8312 showing heteroplasmic T–C transition at position 8306 in muscle mtDNA (top) and its absence from blood mtDNA (bottom). (B) Comparison of the *MTTK* gene in different species. The base pair involving the nucleotide at position 8306 (and 8312) is highly conserved throughout species as shown by the boxes. The mutant base in our patient (Pa.) is underlined in bold. The DNA sequences are human (Hu), bovine (Bo), rat (Ra), murine (Mo), *Xenopus laevis* (Xe), chicken (Ch) and cod (Co). (C) Predicted two-dimensional structure of human mitochondrial $tRNA^{Lys}$, showing the mutation in the D-stem and the affected T–A base pair. The cloverleaf structure of human mitochondrial $tRNA^{Lys}$ was obtained from the website http://mamit-trna.u-strasbg.fr.