Journal of the Neurological Sciences 303 (2011) 142–145

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

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

Short communication

Progressive mitochondrial myopathy, deafness, and sporadic seizures associated with a novel mutation in the mitochondrial tRNASer$^{(AGY)}$ gene

Elena Cardaioli [a], Edoardo Malfatti [a], Paola Da Pozzo [a], Gian Nicola Gallus [a], Maria Alessandra Carluccio [a], Alessandra Rufa [a], Nila Volpi [b], Maria Teresa Dotti [a], Antonio Federico [a,*]

[a] Department of Neurological, Neurosurgical and Behavioural Sciences, University of Siena, Siena, Italy
[b] Department of Biomedical Sciences, Section of Anatomy and Histology, University of Siena, Italy

ARTICLE INFO

Article history:
Received 26 October 2010
Received in revised form 20 December 2010
Accepted 21 December 2010
Available online 22 January 2011

Keywords:
Encephalomyopathies
Mitochondrial DNA
tRNA$^{Ser(AGY)}$
Heteroplasmy
Single fibres studies
Pathogenicity
Muscle biochemical analysis

ABSTRACT

We sequenced the mitochondrial genome from a patient with progressive mitochondrial myopathy associated with deafness, sporadic seizures, and histological and biochemical features of mitochondrial respiratory chain dysfunction. Direct sequencing showed a heteroplasmic mutation at nucleotide 12262 in the tRNASer$^{(AGY)}$ gene. RFLP analysis confirmed that 63% of muscle mtDNA harboured the mutation, while it was absent in all the other tissues. The mutation is predicted to influence the functional behaviour of the aminoacyl acceptor stem of the tRNA. Several point mutations on mitochondrial tRNA genes have been reported in patients affected by encephalomyopathies, but between them only four were reported for tRNASer$^{(AGY)}$.

1. Introduction

Mitochondrial DNA (mtDNA) mutations are important causes of human genetic diseases and are associated with an impressive spectrum of different clinical presentations [1]. Tissues heavily reliant on oxidative metabolism, such as skeletal muscle, brain and heart, are the most affected. However, virtually any organ or tissue in the body may be affected and the disorders can be multisystemic (mitochondrial encephalomyopathies) or confined to a single tissue [2].

To date, more than 200 pathogenic mtDNA mutations have been identified, with mutations involving tRNA appearing to be the most common (http://www.mitomap.org). This can be explained by the essential role of tRNAs in the synthesis of proteins involved in energy metabolism. Interestingly, some tRNAs appear preferentially to be affected, for example, the tRNA for leucine(UUR), lysine, and isoleucine. Mutations in the other tRNA genes are more rarely reported in mitochondrial disorders [3]. In particular, the tRNA for serine(AGY) gene is one of the less affected, with only four mutations reported as pathogenic.

Here we report an adult patient with progressive mitochondrial myopathy associated with deafness, sporadic seizures, EEG changes and periventricular white matter abnormalities at MRI. Morphological, biochemical and molecular studies led us to identify a novel heteroplasmic mutation in the mitochondrial tRNA$^{Ser(AGY)}$ gene.

2. Case report

The patient, a 58-year-old woman was born from healthy unrelated parents. The medical family history was negative for genetic disorders. She was admitted to our hospital for weakness and elevation of serum creatine kinase (>1000 UI/l, n.v. <140). Previous clinical data suggested hypothyroidism, arterial hypertension, Q–T interval prolongation, and sporadic seizures from the age of 53. Neurological examination revealed bilateral muscle weakness and hypotrophy of upper limbs. Venous lactic acid was lightly increased (1.6 mmoli/l; normal value: 0.3–1.3 mmol/l); audiometric examination revealed mild neurosensorial deafness; EEG showed slow left temporal focal activity, with spike-waves abnormalities. EMG showed myopatic pattern. Brain Magnetic Resonance Imaging (MRI) revealed hyperintense T2 signal in the cerebral white matter bilaterally (Fig. 1a). Echocardiography evidenced mitral valve prolapse, resulting in moderate degree of valvular insufficiency. A muscle biopsy was performed showing cytochrome c oxidase (COX) deficiency and 6% of ragged-red fibres (Fig. 1b–d). Muscle biochemical analysis [4] revealed deficiency of complex I and IV of the respiratory chain (Fig. 2a). The muscle biopsy of the only patient's son was histochemically and biochemically normal.

* Corresponding author. Department of Neurological, Neurosurgical and Behavioural Sciences, University of Siena, Viale Bracci 2, 53100 Siena, Italy. Tel.: +39 0577 585763x60; fax: +39 0577 40327.
E-mail address: federico@unisi.it (A. Federico).

0022-510X/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jns.2010.12.020

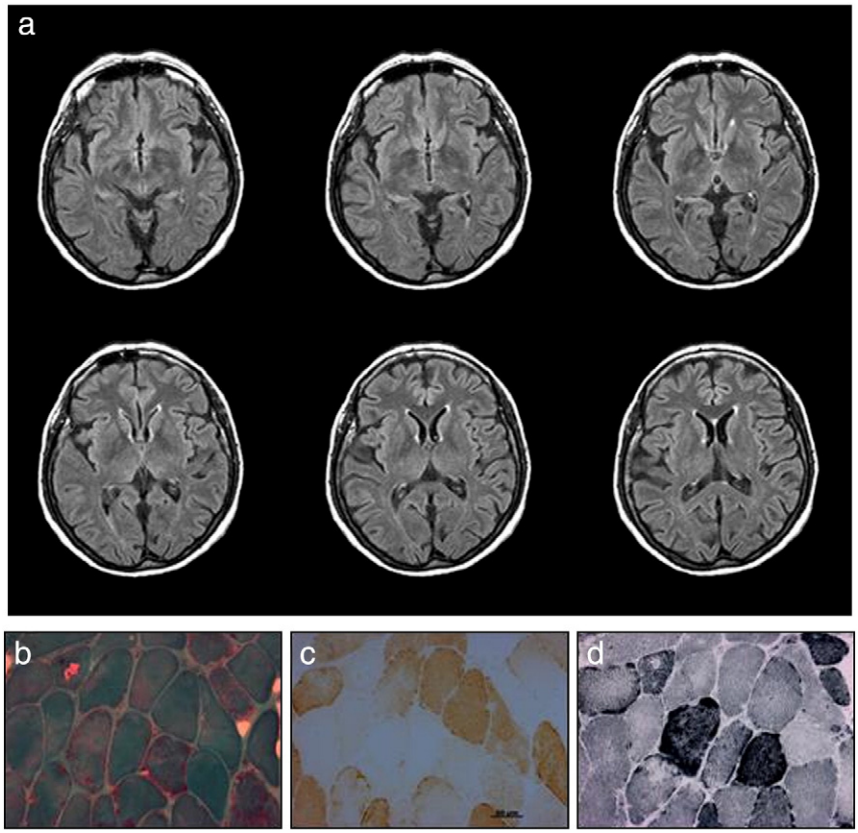

Fig. 1. a) T2-weighted brain MRI scans of the proband, showing high intensity areas in the deep white matter bilaterally. b), c), and d) Serial sections of skeletal muscle: (b) ragged-red fibres and a vacuolated fibre in trichrome stain; (c) RRFs are substantially COX negative; and (d) SDH surcharge in some of the fibres.

2.1. Molecular genetic study

Total DNA was extracted from skeletal muscle, lymphocytes, mouth and urinary tract epithelium and hair roots of the proband; from muscle, lymphocytes, and urinary tract epithelium of the son; from lymphocytes, and urinary tract epithelium of the sister, using QIAmp DNA Mini kits (Qiagen). The whole mitochondrial genome, amplified by PCR from muscle DNA and investigated by sequence analysis, didn't reveal any known mutation in different mt-tRNA genes, while showed the novel C12262A variation on the $tRNA^{Ser(AGY)}$ gene (Fig. 2b). This mutation is in heteroplasmic state and destroys G–C base coupling in the aminoacyl acceptor stem of the $tRNA^{Ser(AGY)}$ molecule (Fig. 2c). Although the C at position 12262 is not highly conserved during evolution, the nucleotide at this particular position is invariably complementary to the nucleotide at position 12209 in various species, resulting in a base pair that is highly conserved throughout evolution (Fig. 3a). Since there is no restriction site that can distinguish mutant from normal DNA, we modified an oligonucleotide that created a restriction site for the enzyme *BclI* on mutant mtDNA. The oligonucleotides used were: "forward" 12228–12261: TAACTCATGCCCCCATGTC-TAACAACATGGCTTG (modified nucleotide underlined); "reverse" 12387–12406: GGGAATTAGGGAAGTCAGGG. The mutation, if present, allowed *BclI* to cut the mutated mtDNA into two fragments of 34 and 144 bp.

PCR-RFLP analyses, and optical densitometry (using the Biorad Gel Doc 2000 image analyzer) of ethidium-bromide stained 3% MS gels showed that 63% of muscle mtDNA carried the mutation, which was absent from mtDNA extracted from lymphocytes, mouth and urinary tract epithelium and hair roots of the proband, as well as in any of the accessible tissues from the patient's healthy sister and son (Fig. 3b). The mutation, never hitherto reported in literature, was not found in 110 Italian patients with different encephalomyopathies.

Single fibre PCR analysis (Fig. 3c) showed a wide variability in the percentage of mutant mtDNAs between the COX deficient fibres and the COX normal fibres. This difference was statistically significant ($P = 1.05\ E{-}06$, 2-tailed *t* test).

3. Discussion

The patient presented with progressive mitochondrial myopathy associated with deafness, and sporadic seizures, which were suggestive of an underlying mitochondrial abnormality. This was confirmed by a muscle biopsy, that revealed ragged-red fibres and a mosaic of COX positive and COX deficient fibres, features consistent with a pathogenic mitochondrial tRNA mutation. Enzymatic investigations in muscle showing decreased activities of complexes I and IV were highly suggestive for a mitochondriopathy, too.

Direct sequencing of the entire coding sequence of the mitochondrial genome from the proband's skeletal muscle revealed a novel C12262A mutation in the aminoacyl acceptor stem of the $tRNA^{Ser(AGY)}$ gene.

Some tRNA genes appear to be frequently affected, such as the $tRNA^{Leu(UUR)}$ gene, while only four different mutations in the $tRNA^{Ser(AGY)}$ have been described. They were associated with chronic intestinal pseudo-obstruction with myopathy and ophtalmoplegia [5], diabetes mellitus with deafness and retinitis pigmentosa with progressive sensorineural hearing loss [6,7], MELAS-like phenotype [8], and non-syndromic hearing impairment [9].

Our mutation fulfils accepted criteria for pathogenicity [10]. First, the mutation has not previously been described in the literature as a neutral polymorphic variant (http://www.mitomap.org; http://www.genpat.uu.se/mtDB/), nor it was detected in 110 disease controls. Second, it was heteroplasmic and present at high level in a postmitotic tissue (skeletal muscle) while it was absent in mitotic cells (buccal