Biochemical and Biophysical Research Communications 500 (2018) 158–162

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Myoclonus epilepsy, retinitis pigmentosa, leukoencephalopathy and cerebral calcifications associated with a novel m.5513G>A mutation in the *MT-TW* gene

Elena Cardaioli [a, *], Andrea Mignarri [a], Teresa Anna Cantisani [b], Alessandro Malandrini [a], Claudia Nesti [c], Anna Rubegni [c], Niccola Funel [d], Antonio Federico [a], Filippo Maria Santorelli [c], Maria Teresa Dotti [a]

[a] Department of Medicine, Surgery and Neuroscience, University of Siena, Viale Bracci 2, 53100, Siena, Italy
[b] Perugia Hospital, Neurophysiopathology Unit, Azienda Ospedaliera di Perugia, S. Andrea delle Fratte, 06156 Perugia, Italy
[c] Molecular Medicine, IRCCS Stella Maris, Via dei Giacinti 2, 56128, Pisa, Italy
[d] Department of Translational Research & The New Technologies in Medicine & Surgery, University of Pisa, Via Paradisa 2, 56124, Pisa, Italy

ARTICLE INFO

Article history:
Received 23 March 2018
Accepted 2 April 2018
Available online 13 April 2018

Keywords:
Mitochondrial disease
mtDNA
$tRNA^{Trp}$
MT-TW gene
New mutation

ABSTRACT

We sequenced the mitochondrial genome from a 40-year-old woman with myoclonus epilepsy, retinitis pigmentosa, leukoencephalopathy and cerebral calcifications. Histological and biochemical features of mitochondrial respiratory chain dysfunction were present. Direct sequencing showed a novel heteroplasmic mutation at nucleotide 5513 in the *MT-TW* gene that encodes $tRNA^{Trp}$. Restriction Fragment Length Polymorphism analysis confirmed that about 80% of muscle mtDNA harboured the mutation while it was present in minor percentages in mtDNA from other tissues. The mutation is predicted to disrupt a highly conserved base pair within the aminoacyl acceptor stem of the tRNA. This is the 17° mutation in *MT-TW* gene and expands the known causes of late-onset mitochondrial diseases.

1. Introduction

Around 200 pathogenic point mutations and large-scale rearrangements have been identified in mitochondrial DNA (mtDNA) associated with a wide variety of human diseases.

tRNAs are responsible for more than half of pathogenic mtDNA point mutations, this being explained by the essential role of these molecules in the synthesis of proteins involved in mitochondrial oxidative phosphorylation. Whilst affecting a similar substrate, different tRNA mutations give rise to widely differing phenotypes, ranging from isolated organ-specific diseases to multisystem disorders [1].

In this report, we describe a patient who presented with myoclonus epilepsy, retinitis pigmentosa, leukoencephalopathy and cerebral calcifications.

2. Materials and methods

2.1. Case report

The proband is a 40-year old Italian woman, single daughter of non-consanguineous parents. She came to our attention for a six-year history of myoclonic seizures, previously treated with valproate which was ineffective and caused important side effects (asthenia, cramps, generalized malaise). She had been diagnosed with retinitis pigmentosa at age 35. Her past history was unremarkable. Neurological examination showed only mild dysarthria, and neuropsychological tests did not reveal abnormalities. Routine blood tests (including serum lactate and thyroid hormones), cardiologic examination, audiometry, and electromyography were normal. Electroencephalogram uncovered widespread paroxysmal abnormalities. Brain CT and MRI showed calcification of basal ganglia, thalami, and dentate nuclei, and leukoencephalopathy with diffuse and focal non enhancing lesions involving periventricular and subcortical white matter (Fig. 1a). Cervical MRI was normal. Due to the presence of marked brain calcification, we performed a complete evaluation of phospho-calcium metabolism

* Corresponding author.
E-mail address: cardaioli@unisi.it (E. Cardaioli).

https://doi.org/10.1016/j.bbrc.2018.04.009
0006-291X/

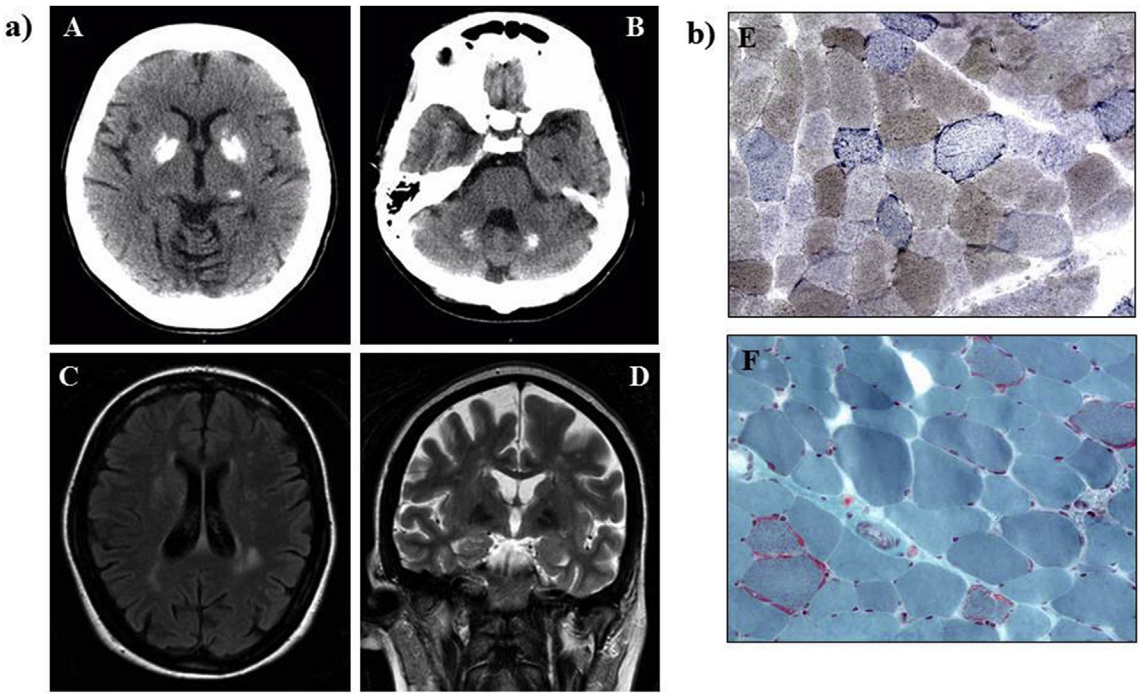

Fig. 1. a) Neuroimaging study of the brain including axial CT (A, B), axial FLAIR MRI (C), and coronal T2 (D) MRI shows: calcification of basal ganglia (A, D), left thalamus (A), and cerebellar dentate nuclei (B); periventricular and subcortical white matter disease (C, D). **b)** Histochemical investigations in the patient. Muscle biopsy (light microscopy × 300): (E) COX-SDH stain showing COX-negative fibres; (F) Modified Gomori's trichrome staining showing ragged red fibres. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

on serum and urines, parathyroid hormone assessment, and molecular analysis of the genes causing primary familial brain calcification (*SLC20A2*, *PDGFRB*, *PDGFB*, and *XPR1*): all these examinations resulted negative. Treatment with levetiracetam 500 mg bid was started and sharply reduced myoclonic seizures and electroencephalographic abnormalities.

Since clinic-anamnestic, neuroradiological, and bioptic findings (reported below) pointed to a mitochondrial encephalomyopathy, we performed analysis of mitochondrial DNA.

2.2. Morphological and biochemical analyses

Skeletal muscle biopsy was performed according to standard procedures.

Morphological analysis of skeletal muscle and biochemical assays of the mitochondrial respiratory chain enzymes activity by standard spectrophotometric techniques in muscle homogenate were carried out as previously described [2].

Specific activities of each complex were normalised to that of citrate synthase, an index of mitochondrial mass.

2.3. Molecular analysis

Total DNA was extracted from various tissues (muscle -only from the proband-, blood, buccal swabs, hair roots and urinary sediment) of the proband, and her mother with the QIAmp DNA Mini kit (Qiagen, Hilden, Germany).

Direct sequencing of the whole mitochondrial genome from skeletal muscle was performed on an automatic sequence analyzer, as reported earlier [3].

PCR-RFLP analysis was used to quantify the percentage of the m.5513G > A mutation on different tissues and on single muscle fibres, isolated from serial 10-μm thick transversal sections obtained from frozen muscle and stained for cytochrome C oxidase (COX) activity using standard methods. Two independent laser capture microdissection (LCM) of COX positive and COX negative muscle fibres were performed under direct microscopic visualization (LMD 6500, Leica Microsystems, Germany).

Digestion products were electrophoresed on a 12% acrylamide gel, and quantification was performed with ImageJ software (http://rsbweb.nih.gov/ij).

2.4. Analysis of mtDNA variants

The patient sequence was compared to the revised Cambridge reference sequence (GenBank: NC_012920) and to mtDNA sequences available in the Mitomap database (http://www.mitomap.org) and in GiiB-JST mtSNP database (http://mtsnp.tmig.or.jp). Variants were considered rare if they occurred <10 times in the Mitomap database among 42,616 sequences. Conservation of nucleotides among species was evaluated using Mamit tRNA (http://mamit-trna.u-strasbg.fr). The pathogenicity of m.5513G > A mutation was also assessed by means of a multifactorial probability-based classification method PON-mt-tRNA that predicts pathogenicity of single nucleotide substitutions in human mitochondrial tRNAs [4].

3. Results

The proband's muscle biopsy contained around 20% ragged red fibers (RRFs), and 50% COX-negative fibres (Fig. 1b).

Biochemical analysis of the patient's muscle homogenate showed a reduction of about 50% of complexes I and IV (Table 1), which supported the diagnosis of mitochondrial disease.

Sequencing of muscle mtDNA revealed a heteroplasmic G > A transition at position 5513 in the $tRNA^{Trp}$ (encoded by the *MT-TW* gene) (Fig. 2c). This base change occurred once in the Mitomap database among 42,616 sequences and it has not be seen in the