Molecular Genetics and Metabolism Reports 6 (2016) 70–73

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

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

Case Report

A novel mitochondrial tRNA^{Ala} gene variant causes chronic progressive external ophthalmoplegia in a patient with Huntington disease

Massimiliano Filosto [a,*], Gaetana Lanzi [b], Claudia Nesti [c], Valentina Vielmi [a], Eleonora Marchina [d], Anna Galvagni [a], Silvia Giliani [b], Filippo M. Santorelli [c], Alessandro Padovani [a]

[a] Clinical Neurology, Section for Neuromuscular Diseases and Neuropathies, University Hospital "Spedali Civili", Brescia, Italy
[b] Institute of Molecular Medicine "A. Nocivelli", University Hospital "Spedali Civili", Brescia, Italy
[c] Unit of Molecular Medicine, IRCCS Stella Maris, Pisa, Italy
[d] Division of Biology and Genetics, Department of Biomedical Sciences and Biotechnology, University of Brescia, Italy

ARTICLE INFO

Article history:
Received 7 February 2016
Accepted 7 February 2016
Available online 27 February 2016

Keywords:
Mitochondrial diseases
CPEO
Huntington disease
mtDNA
tRNA

ABSTRACT

Chronic progressive external ophthalmoplegia is a mitochondrial disorder usually caused by single or multiple mitochondrial DNA (mtDNA) deletions and, more rarely, by maternally inherited mtDNA point mutations, most frequently in tRNA genes (*MTT*).

We report on a patient presenting with a progressive eyelid ptosis with bilateral ophthalmoparesis, dysphagia, dysphonia and mild proximal limb weakness associate with a mild movement disorder characterized by abnormal involuntary movements involving head and limbs, imbalance and gait instability.

Muscle biopsy demonstrated the presence of ragged red fibers and several cytochrome-C-oxidase negative fibers. Molecular analysis showed the novel m.5613T > C heteroplasmic mutation in the mitochondrial tRNA^{Ala} gene (*MTTA*) which disrupts a conserved site and fulfills the accepted criteria of pathogenicity. Moreover, a 38 CAG trinucleotide repeat expansion was found on the huntingtin gene, thus configuring a singular CPEO/"reduced penetrance" Huntington disease "double trouble".

With this novel *MTTA* point mutation, we extend the spectrum of provisional pathogenic changes in this gene, which is a very rare site of pathogenic mutation, and confirm that clinical expression of these mutations is hardly ever heterogeneous, including myopathy and CPEO.

Mitochondrial involvement is an emerging key determinant in the pathogenesis of Huntington disease and it is well known that mutant huntingtin influences the mitochondrial respiratory complexes II and III. A synergist effect of the *HTT* and *MTTA* mutations on respiratory chain function may be hypothesized in our patient and should be regarded as a spur for further studies on the mtDNA/*HTT* reciprocal interactions.

1. Introduction

Mitochondrial DNA (mtDNA) mutations are associated with a wide spectrum of disorders involving different tissues, particularly brain and muscle [1, 2].

Chronic progressive external ophthalmoplegia (CPEO) is a classical mitochondrial disorder characterized by bilateral progressive ptosis and ophthalmoplegia. These ocular features can develop either in isolation or in association with other prominent neurological deficits. Molecularly, CPEO can be classified into three distinct genetic subgroups depending on whether patients harbour single large-scale mtDNA deletions or multiple mtDNA deletions secondary to a nuclear mutation disrupting mtDNA replication and repair or point mutations in the mitochondrial genome.

Patients harbouring a single mtDNA deletion are sporadic and the mutation is thought to arise in oogenesis or in early embryonic development. In contrast, multiple deletions of mtDNA are observed in autosomal dominant or recessive forms of CPEO [2–7]. More rarely, CPEO is maternally inherited and due to mtDNA point mutations, most frequently in tRNA genes (*MTT*) [8].

Huntington disease (HD) is an autosomal dominant, adult-onset, progressive neurodegenerative disease characterized by abnormal movements, cognitive impairment and psychiatric disorders [9]. It is caused by an expanded CAG repeat in the gene *HTT* encoding the huntingtin on chromosome 4, resulting in an expanded N-terminal polyglutamine stretch in the protein, and it is characterized by a progressive atrophy of the striatum as well as cortical and other extrastriatal structures [10].

Here, we report on the identification of a novel point mutation in the *MTTA* gene in a patient affected with a sporadic CPEO. She also presents

* Corresponding author at: Clinical Neurology, University Hospital "Spedali Civili", Pz.le Spedali Civili 1, 25100 Brescia, Italy.
E-mail address: massimiliano.filosto@unibs.it (M. Filosto).

http://dx.doi.org/10.1016/j.ymgmr.2016.02.001

a "reduced penetrance" Huntington disease (HD). This very unusual "double trouble" condition represents the first reported case of a concomitance of these two disorders.

2. Patient and methods

2.1. Patient

A 70-year-old woman presented with progressive eyelid ptosis, bilateral ophthalmoparesis, dysphagia, dysphonia, mild proximal limb weakness, numbness and bilateral deafness since age of 55.

By the age of 64 she noticed some abnormal involuntary movements involving head and limbs and imbalance and gait instability. Mini Mental State Examination was rated 20/30. Family history was unremarkable.

Neurological examination showed bilateral eyelid ptosis, severe ophthalmoparesis in all the directions of gaze and mild involuntary choreiform movements of the head and distal limbs.

Blood and urine assays, including resting blood lactate, were normal except for serum CK levels that were repeatedly 2–3 times the normal values. Pure tone audiometry showed bilateral sensorineural deafness. EMG recording showed low-amplitude and short duration of motor unit potentials in the four limbs. Electroencephalography was normal as well as electrocardiography and echocardiography. Brain MRI showed cerebellar atrophy that affects more severely the superior and inferior semilunar lobules and the vermis. There is also a widespread mild brain cortical atrophy and an extrinsic ocular muscles atrophy.

A biopsy of vastus lateralis muscle was performed and urine and blood samples were collected.

2.2. Methods

Morphological study of the muscle biopsy were done as described [11]. Mitochondrial respiratory chain complex activities were assayed according to established spectrophotometric methods and expressed as nmol/min/mg of protein [12]. DNA was extracted from muscle, blood and urine samples by Puregene DNA purification Kit (Gentra Systems, MN, USA).

Southern Blot was carried out with standardized procedures [8]. The sequence of the entire mtDNA was performed with suitable nucleotide primers as reported [13]. PCR-RFLP analysis was used to quantify the percentage of the m.5613T > C mutation. In brief, a fragment between mtDNA nucleotides 5544 and 5739 was amplified, digested with endonuclease *Sfa*NI, and electrophoresis was performed on a 3% agarose gel. The enzyme cuts the wild-type DNA into 2 fragments, whereas the presence of the mutation abolishes the restriction site.

Isolation of single muscle fibers was performed on serial 10-μm thick transversal sections obtained from frozen muscle and stained for COX activity using standard methods; after fixation and dehydratation, two independent laser capture microdissection (LCM) of COX positive and COX negative muscle fibers were performed under direct microscopic visualization (PALM Robot Microbeam, PALM MicrolaserTechnologies AG, Munich, Germany). Isolated DNA (using QIAmp DNA Micro Kit, QIAGEN, Hilden, Germany) was used for PCR-RFLP as described above.

Molecular test for Huntington disease was performed as previously described [10].

3. Results

Muscle biopsy showed few atrophic fibers, many fibers with subsarcolemmal accumulation of mitochondria, some ragged red fibers (RRFs) (Fig. 1A) and several cytochrome C oxidase (COX)-negative fibers (Fig. 1B). All RRFs were COX-negative. No major rearrangements in mitochondrial DNA were detected by Southern Blot (data not shown).

Sequencing of the whole mtDNA in muscle revealed a heteroplasmic m.5613T > C transition in the tRNA Alanine gene (*MTTA*) which disrupts a highly conserved residue through the species in the V-region of the molecule (Fig. 2A, C and D). This change was absent in one hundred and fifty unrelated muscle controls and in large mitochondrial variation databases (Mitomap, www.mitomap.org; Human Mitochondrial Genome Database, www.mtdb.igp.uu.se). The mutation, which accounted for 96% of the total mitochondrial genomes in muscle (Fig. 3A), was no detectable in blood and urine from the patient and her two sons (Fig. 2B).

PCR-RFLP analysis on COX-positive and COX-deficient single fibers clearly showed highest levels of the mutation segregating with the biochemical defect (Fig. 3B). Biochemical assay showed a partial reduction of complexes I and IV (30% and 24% respectively) and a severe impairment of complex II + III activity (80%) (Table 1); citrate synthase was increased, suggesting mitochondrial proliferation. An expansion of 38 CAG repeats was detected in the coding region of *HTT* gene, which falls in the range of intermediate/reduced penetrance alleles (data not shown).

4. Discussion

Both large rearrangements and point mutations in mtDNA have been described in association with sporadic CPEO [1, 8]. In our patient we identified the novel heteroplasmic m.5613T > C mutation in the *MTTA* gene that we consider to be pathogenic for several reasons: first, it is consistent with the histochemical and biochemical findings; second, the mutation disrupts a strongly conserved base pair site in the molecule; third, it is heteroplasmic and the mutational load is related to COX deficiency, as shown by single-fiber PCR; fourth, it was absent in 150 unrelated control subjects. Moreover, the m.5613T > C mutation results "definitely pathogenetic" by applying the pathogenicity scoring system for mitochondrial tRNA variations (which summarize functional and biochemical studies, heteroplasmy, segregation, conservation) [14].

The *MTTA* gene is a rare site of pathogenic mutation. The Mitomap dataset (www.mitomap.org) reports four pathogenic mutations in the $tRNA^{Ala}$ gene, two of them associated with CPEO and two with myopathy, all of them having high mtDNA heteroplasmy thresholds [15–18]. More recently, two other mutations in the $tRNA^{Ala}$ gene (m.5631G > A

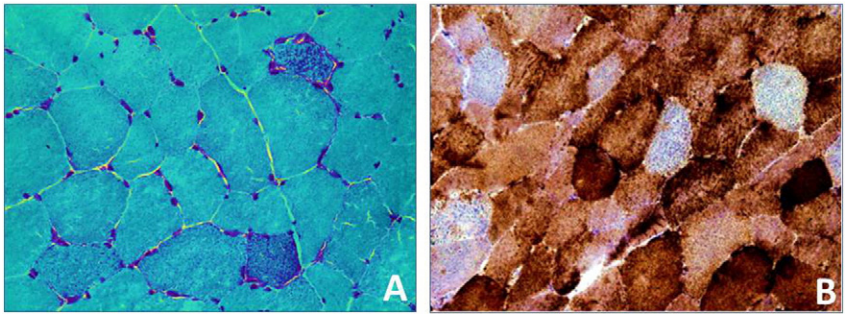

Fig. 1. Muscle biopsy showed mitochondrial abnormalities: A: Modified Gomori's Trichrome staining showing ragged red fibers B: COX-SDH double staining showing several COX-negative fibers