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Short communication

A novel point mutation in the mitochondrial tRNA$^{(Trp)}$ gene produces late-onset encephalomyopathy, plus additional features

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ABSTRACT

Background: Mitochondrial diseases due to mitochondrial tRNA genes mutations are usually multisystem disorders with infantile or adult onset.
Objective: To identify the molecular defect underlying a mitochondrial encephalomyopathy.
Methods/Patients: Case report of a 51 year-old woman presenting with late-onset myoclonic epilepsy plus additional features. Proband's mother presented hypothyroidism and diabetes.
Results: Muscle biopsy showed mitochondrial changes. Respiratory chain activities were reduced. The novel G5538A mutation was identified in different tissues DNAs from the proband and from her mother.
Conclusion: We were able to identify a novel mtDNA tRNA$^{(Trp)}$ gene pathogenic mutation.

1. Introduction

Mitochondrial encephalomyopathies (MIM 251900) include a diverse group of disorders of mitochondrial function [1]. More than 50% of pathogenic mtDNA mutations are concentrated in tRNA genes [2]. We describe a novel, maternally inherited mtDNA point mutation in the tRNA$^{(Trp)}$ gene, associated with unusual clinical features. The proband developed late-onset encephalomyopathy, her mother had hypothyroidism and diabetes mellitus.

2. Methods

2.1. Patients

The proband, a 51-year-old woman, had a normal childhood, and two uneventful pregnancies at ages 20 and 27. At age 40, she developed myoclonic jerks. At age 42, she manifested major weakness and hair loss; blood tests revealed hypothyroidism. Retinal examination showed pigmentary changes. Visual evoked response was delayed in the left eye. EMG showed myopathic changes. Brain CT showed calcification in the left lenticular nucleus (Fig. 1B). EEG showed a burst of high amplitude 4–5 Hz multispike and wave activity. Skeletal muscle biopsy revealed RRFs (Ragged Red Fibers) and cytochrome *C* oxidase (COX)-negative fibers (Fig. 2A). The proband's mother, aged 74, had a history of hypothyroidism and diabetes mellitus; neurological examination was unremarkable. Skeletal muscle biopsy revealed mitochondrial abnormalities (Fig. 2B). The proband's daughter and son were unavailable for study.

2.2. Analysis

Morphological analysis of skeletal muscle and biochemical assays of individual respiratory chain complexes were carried out on muscle homogenate, as described [3]. Enzyme activities were normalized to that of citrate synthase, an index of mitochondrial mass.

Total DNA was extracted from different tissues by standard procedures.

The entire mtDNA molecule was PCR-amplified and direct sequencing was performed as reported [4]. Screening for the G5538A mutation of mtDNA was done by (RFLP)-PCR analysis of a 165 bp fragment amplified by modified primers that create a recognition site for the endonuclease *HhaI*. The presence and frequency of G5538A was verified by mismatch RFLP of the PCR product. The oligonucleotides used were FW (nt. 5408–5428) AAAATGACAGTTTAGGTCTAC, and RV modified (nt. 5573–5539) TTAAGTATTGCAACTTACTGAGGGCTTTGAAGGC**G** with one mismatch (in bold). The RV modified primer contains a mismatch at n. 5539 that creates a novel *HhaI* recognition site (nt. 5538–5542 3′ C/GCG 5′). The 165 bp fragment is cleaved by the endonuclease into two fragments of 131 and 34 bp (not shown). The presence of m. G5538A abolishes the *HhaI* site so that mutant mtDNA remains uncut. Digestion products were separated on 4% MS agarose gel. Bands were visualized

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