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Mutational analysis in patients with neuromuscular disorders: Detection of mitochondrial deletion and double mutations in the MT-ATP6 gene

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ABSTRACT

Mitochondrial diseases encompass a wide variety of pathologies characterized by a dysfunction of the mitochondrial respiratory chain resulting in an energy deficiency. The respiratory chain consists of five multi-protein complexes providing coupling between nutrient oxidation and phosphorylation of ADP to ATP. In the present report, we studied mitochondrial genes of complex I, III, IV and V in 2 Tunisian patients with mitochondrial neuromuscular disorders. In the first patient, we detected the m.8392C>T variation (P136S) in the mitochondrial ATPase6 gene and the m.8527A>G transition at the junction MT-ATP6/MT-ATP8 which change the initiation codon AUG to GUG. The presence of these two variations in such an important gene could probably affect the ATP synthesis in the studied patient. In the second patient, we detected several known variations in addition to a mitochondrial deletion in the major arc of the mtDNA eliminating tRNA and respiratory chain protein genes. This deletion could be responsible of an inefficient translation leading to an inefficient mitochondrial protein synthesis in P2.

1. Introduction

Mitochondrial diseases are a clinically heterogeneous group of disorders that arise as a result of dysfunction of the mitochondrial respiratory chain affecting the mitochondrial oxidative phosphorylation. This system is a key functional unit in the mitochondria, and combines electron transport with cell respiration and ATP synthesis to produce energy. The human oxidative phosphorylation system consists of five multi-subunit complexes of which the individual subunits, with the exception of complex II, are encoded either by mitochondrial or nuclear DNA. Consequently, a deficient enzyme activity of one or more of the complexes can be caused by mitochondrial or nuclear DNA mutations leading to mitochondrial disorders [1,2].

Pathogenic mitochondrial DNA (mtDNA) point mutations are associated with a wide range of clinical phenotypes, often involving multiple organ systems but the nervous system and skeletal muscles are mainly involved because of their high dependence on oxidative metabolism [3–5]. In fact, over the past 20 years, mitochondrial dysfunction has been increasingly recognized as responsible for some neuromuscular and neurodegenerative diseases [6]. However, the clinical features are usually extremely heterogeneous because the mitochondrial diseases may involve several tissues with different degrees of severity, and patients may present with a wide range of clinical features in various combinations [7]. The complexity of this clinical and genetic heterogeneity of the mitochondrial disorders makes the genotype–phenotype correlation difficult [4].

Defects in mtDNA may vary from punctual mutations to larger rearrangements such as duplications or deletions [8]. One hundred and sixteen different mtDNA deletions have been recorded in the latest MITOMAP databases (www.mitomap.org) in patients with mitochondrial dysfunction [9], and single mtDNA deletions were reported in third of adult patients presenting with mitochondrial

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disease [10].

In the present report, we screened mitochondrial genes of complex I, III, IV and V in 2 Tunisian patients with mitochondrial neuromuscular disorders. We detected double mitochondrial mutations in patient 1: m.8392C>T (P136S) in the mitochondrial ATPase6 gene and the m.8527A>G transition at the junction MT-ATP6-MT-ATP8. We also detected a deletion in the mitochondrial DNA of patient 2.

2. Patients and methods

2.1. Patients

In this report, we studied 2 unrelated Tunisian patients (P1 and P2) with clinical, radiological, and biochemical presentations suggestive of neuromuscular disorders.

- *Patient 1* (*P1*)

It's a 7-years-old boy born at term to healthy parents after an uneventful pregnancy with a weight birth of 3750 g, a height of 51 cm, a head circumference of 35 cm and an Apgar index of 9/10. He presented a psychomotor retardation, global and axial hypotonia and seizures. His electroencephalogram (EEG) showed significant abundant short flashes. His electromyography (EMG) and his Motor Nerve Conduction Velocity (MNCV) evoked a significant slowdown of motor conduction velocity (MCV). Brain MRI examination showed bilateral abnormalilies signal intensity in the lenticular nucleus and in the bilateral periventricular white matter. It also shows abnormal high signal intensity in the bilateral periventricular white matter and the bilateral dentate nucleus (Fig. 1).

- *Patient 2* (*P2*)

He's a 9-years-old boy born at term to healthy parents after an uneventful pregnancy with a weight birth of 3300 g. He presented with psychomotor retardation, hypotony and seizures since the age of 4 years. Laboratory investigations revealed raised blood pyruvate level (3.8 mmol/l).

Controls

In addition, 200 Tunisian healthy individuals from the same ethnocultural group were tested as controls. These controls should have no personal or family history of any disorder. All individuals (patient and controls) provided informed consent.

2.2. Methods

2.2.1. DNA extraction

After getting informed consent from our patients, the total DNA was extracted from peripheral blood using phenol chloroform standard procedures [11].

2.2.2. PCR amplification

The mitochondrial genes associated with neuromuscular disorders tested in this report were amplified with 8 overlapping fragments covering several genes in complex I, III, IV and V (Table 1). The PCR amplification was carried out in a Perkin–Elmer GeneAmp PCR System 9700 thermal cycler in a final volume of 50 mL using 200 ng DNA, 8 pmol of each primer, 2 mmol/L $MgCl_2$, 500 mmol/L deoxynucleotide triphosphate, 1 × PCR buffer, and 2U Taq DNA polymerase. The conditions for PCR amplification were as follows: initial denaturation at 95 °C for 5 min followed by 35 cycles (1 min at 94 °C; 1 min at 56.5 °C; 1 min at 72 °C), and a final extension at 72 °C for 5 min.

2.2.3. Long-range PCR amplification from peripheral blood

Long-range PCR was performed using Long PCR Enzyme Mix (# K0182) (Fermentas) using 1 pair of primers (Table). A 8.109 Kb fragment was amplified by PCR reaction in a thermal cycler (GenAmp PCR System 9700; Applied Biosystem) using the following primers: 5′ ACGAGTACACCGACTACGGC 3′ and 5′ AGCTTTGGGTGCTAATGGTG 3′ respectively for the forward and the reverse primer.

The PCR amplification was performed using the long-range PCR enzyme mix. The conditions for the PCR reaction were: initial denaturation at 93 °C for 3 min, followed by 10 cycles: 30 s at 93 °C, 30 s at 58.5 °C and 12 min at 68 °C and then 25 cycles at 93 °C for 30 s, 58,5 °C for 30 s, 68 °C for 12 min and 10 s, and a final extension at 68 °C for 11 min. Products were separated on 0.8% agarose gel and visualized with ethidium bromide.

2.2.4. Automatic sequencing

After PCR amplification, each PCR product was purified using NucleoSpin® (MACHEREY-NAGEL) and subsequently analyzed by direct sequencing in an ABI PRISM 3100-Avant automated DNA sequencer using the BigDye Terminator Cycle Sequencing reaction kit v1.1. The resultant sequences were compared with the update Cambridge sequence (GenBank accession number: NC_012920). The blast homology searches were performed using the programs available at the National Center for Biotechnology Information Web site compared with the wild-type sequence.

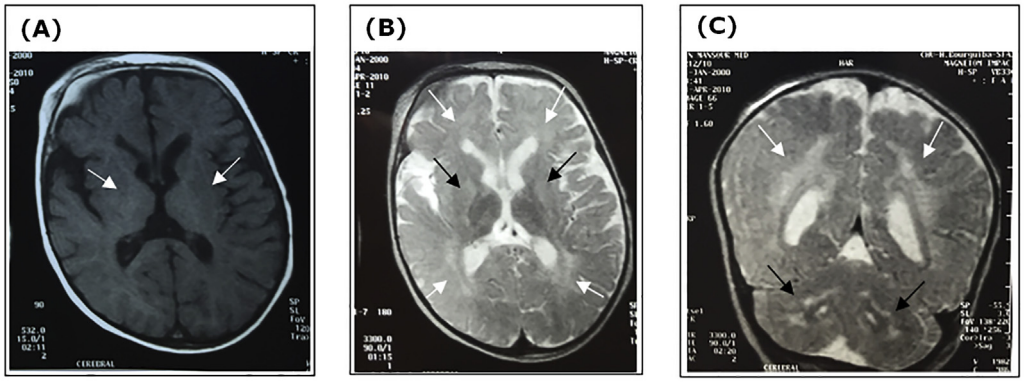

Fig. 1. Brain MRI findings in Patient 1: **(A)** Axial T1-weighted image shows bilateral hypointensity in the lenticular nucleus (White arrows), **(B)** Axial T2 weighted shows an abnormally high signal intensity in the lenticular nucleus (black arrows) and in the bilateral periventricular white matter (White arrows), **(C)** Coronal T2 weighted shows an abnormally high signal intensity in the bilateral periventricular white matter (White arrows) and the bilateral dentate nucleus (black arrow).