Biochemical and Biophysical Research Communications xxx (2016) 1–8

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Mitochondrial DNA triplication and punctual mutations in patients with mitochondrial neuromuscular disorders

Emna Mkaouar-Rebai [a, *], Rahma Felhi [b], Mouna Tabebi [b], Olfa Alila-Fersi [a], Imen Chamkha [a], Marwa Maalej [b], Marwa Ammar [b], Fatma Kammoun [c], Leila Keskes [b], Mongia Hachicha [c], Faiza Fakhfakh [a, **]

[a] Département des Sciences de la Vie, Faculté des Sciences de Sfax, Université de Sfax, Tunisia
[b] Laboratoire de Génétique Moléculaire Humaine, Faculté de Médecine de Sfax, Université de Sfax, Tunisia
[c] Service de pédiatrie, C.H.U. Hedi Chaker de Sfax, Tunisia

ARTICLE INFO

Article history:
Received 22 March 2016
Accepted 26 March 2016
Available online xxx

Keywords:
Mitochondrial mutations
mtDNA
Mitochondrial duplication
m.9157G>A
m.14924T>C
MT-ATP6

ABSTRACT

Mitochondrial diseases are a heterogeneous group of disorders caused by the impairment of the mitochondrial oxidative phosphorylation system which have been associated with various mutations of the mitochondrial DNA (mtDNA) and nuclear gene mutations. The clinical phenotypes are very diverse and the spectrum is still expanding. As brain and muscle are highly dependent on OXPHOS, consequently, neurological disorders and myopathy are common features of mtDNA mutations. Mutations in mtDNA can be classified into three categories: large-scale rearrangements, point mutations in tRNA or rRNA genes and point mutations in protein coding genes. In the present report, we screened mitochondrial genes of complex I, III, IV and V in 2 patients with mitochondrial neuromuscular disorders. The results showed the presence the pathogenic heteroplasmic m.9157G>A variation (A211T) in the MT-ATP6 gene in the first patient. We also reported the first case of triplication of 9 bp in the mitochondrial NC7 region in Africa and Tunisia, in association with the novel m.14924T>C in the MT-CYB gene in the second patient with mitochondrial neuromuscular disorder.

1. Introduction

Mitochondria perform crucial roles in many biochemical processes. They generate ATP by oxidative phosphorylation and participate in numerous metabolic pathways such as citric acid cycle, fatty acid degradation, urea cycle, and the biosynthesis of lipids and amino acids [1].

The human mitochondrial DNA (mtDNA) is a double-stranded DNA molecule of about 16.6 kb. Most information is encoded on the heavy (H) strand, with genes for two rRNAs, 14 tRNAs, and 12 polypeptides. The light (L) strand codes for eight tRNAs and a single polypeptide. Almost all the mtDNA is coding except for one regulatory region (D-loop: the displacement loop) and few non coding sequences (NC) limited to a few bases [2].

All 13 protein products are constituents of the enzyme complexes of the oxidative phosphorylation system (OXPHOS) which produce most of the energy needed by human cells by coupling energy of the electrochemical proton gradient generated by the respiratory complexes (complex I, III and IV) across the mitochondrial inner membrane to produce ATP through ATP synthase (complex V). Impairments in OXPHOS lead to mitochondrial disorders which are clinically and genetically heterogeneous [3,4]. This heterogeneity stems from the dual genetic origin of the respiratory complexes. Indeed, respiratory complexes (I, III, IV and V) depend on two genomes; mitochondrial genome contributes with 13 polypeptides in the assembly of respiratory complexes whereas 74 proteins constituents of these complexes are nuclear encoded [5].

The clinical presentation of those mitochondrial disorders is extremely heterogeneous, and combined with the large number of causal genetic and environmental factors, makes their diagnosis challenging [6,7]. As brain and muscle are highly dependent on OXPHOS, neurological disorders and myopathy are the common

* Corresponding author. Département des Sciences de la Vie, Faculté des Sciences de Sfax, Route Soukra, Km 3, Sfax, Tunisia.
** Corresponding author. Département des Sciences de la Vie, Faculté des Sciences de Sfax, Route Soukra, Km 3, Sfax. Tunisia.
E-mail addresses: emna.mkaouar@gmail.com (E. Mkaouar-Rebai), faiza.fakhfakh02@gmail.com (F. Fakhfakh).

http://dx.doi.org/10.1016/j.bbrc.2016.03.126
0006-291X/

features of mtDNA mutations [8]. Since 1988, more than 270 point mutations have been described in the human mitochondrial DNA leading to inadequate ATP production and resulting to an organ dysfunction that will depend on the tissue's energy requirement [9–11].

Mutations in mtDNA can be classified into three categories: large-scale rearrangements, point mutations in tRNA or rRNA genes and point mutations in protein coding genes. These mutations could affect protein translation causing multiple deficiencies of respiratory chain complexes or result in specific deficiencies of certain respiratory chain complexes [8]. Large-scale mtDNA rearrangements occur as deletions, which remove multiple tRNA and protein coding genes [12], or as duplication, which are dimers of wild-type mtDNA molecules [13]. The translation defect caused by deletions and/or insertions is believed to cause an abnormal expression of almost all mitochondrial-encoded proteins [14].

In the present report, we screened mitochondrial genes of complex I, III, IV and V in 2 patients with mitochondrial neuromuscular disorders. The results showed the presence of the heteroplasmic m.9157G>A mutation in the MT-ATP6 gene the first patient 1 and a mitochondrial triplication of 9 bp with the novel homoplasmic m.14924T>C variation in the MT-CYB gene in the second patient.

2. Patients and methods

2.1. Patients

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

- *Patient 1 (P1)*

He's a 6-years-old boy born at term to healthy parents after an uneventful pregnancy with a weight birth of 3500 g. He suffered from psychomotor retardation, axial and peripheral hypotonia, and seizures. Laboratory investigations revealed elevated blood lactate level = 3.62 mmol/L (normal value < 2 mmol/L) and ammonia = 73.1 mmol/l (normal value < 40 μmol/L). Brain MRI examination showed an important cortical atrophy and his electroencephalogram (EEG) showed right occipital sporadic bridges. The electroretinography (ERG) revealed electrical signs of retinopathy.

- *Patient 2 (P2)*

He's a 7-years-old boy delivered by caesarean section to healthy parents after an uneventful pregnancy. He has a weight birth of 2250 g with a height of 49 cm, a head circumference of 34 cm and an Apgar index of 9/10. Since the first months of life, he suffered from neurodevelopmental and psychomotor delay with an atrophy of the lower limbs. Laboratory investigations revealed raised blood lactate level (3.3 mmol/L). His osteo tendon reflexes were abolished and he was unable to stand up and to walk.

2.1.1. Controls

In addition, 200 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 [15].

2.2.2. PCR amplification

We amplified 8 overlapping mitochondrial fragments covering several genes in complex I, III, IV and V which are associated with neuromuscular disorders (Table 1). The PCR amplification reaction was carried out in a Perkin-Elmer GeneAmp PCR System 9700 thermal cycler as following: 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. The reaction was performed in a final volume of 50 μL using 200 ng DNA, 8 pmol of each primer, 2 mmol/L $MgCl_2$, 500 mmol/L dNTP, 1 x PCR buffer, and 1 U Taq DNA polymerase.

2.2.3. Long-range PCR amplification from peripheral blood

Long-range PCR was performed using Long PCR Enzyme Mix (#K0182) (Fermentas). A 10.162 Kb fragment was amplified in a thermal cycler (GenAmp PCR System 9700; Applied Biosystem) using the following primers 5′ ACTAATTAATCCCCTGGCCC 3′ 5′ GAGTGGTTAATAGGGTGATAG 3′ respectively for the forward and the reverse primer.

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. PCR products were separated on 0.8% agarose gel.

2.2.4. Direct sequencing

After PCR amplification, each PCR product was purified 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.

2.2.5. The sequence alignment

The sequence alignment of the mitochondrial genes was performed using the ClustalW program (http://www.ebi.ac.uk/Tools/msa/clustalw2/).

2.2.6. Prediction of possible impact of an amino acid substitution

The assessment of the possible impact of an amino acid substitution on the three-dimensional protein structure and the possible effect of the mtDNA change on protein function was performed using 2 programs:

- PolyPhen-2 (Polymorphism Phenotyping v2) which is a tool predicting possible impact of an amino acid substitution on the structure and function of a human protein using straightforward physical and comparative considerations (http://genetics.bwh.harvard.edu/pph2/index.shtml) [16,17].
- PROVEAN (Protein Variation Effect Analyzer) program which predict whether an amino acid substitution or indel mutation has an impact on the biological function of a protein. It's useful to predict whether sequence nonsynonymous variants or indel variants are predicted to be functionally important. Scores of <−2.5 indicate the polymorphism is possibly damaging, and scores of >−2.5 is likely benign (http://provean.jcvi.org/seq_submit.php) [18].

Please cite this article in press as: E. Mkaouar-Rebai, et al., Mitochondrial DNA triplication and punctual mutations in patients with mitochondrial neuromuscular disorders, Biochemical and Biophysical Research Communications (2016), http://dx.doi.org/10.1016/j.bbrc.2016.03.126