Short Communication

A novel variant m.8561C > T in the overlapping region of *MT-ATP6* and *MT-ATP8* in a child with early-onset severe neurological signs

Konstantina Fragaki[a], Annabelle Chaussenot[a], Valerie Serre[b], Cecile Acquaviva[c], Sylvie Bannwarth[a], Cecile Rouzier[a], Brigitte Chabrol[d], Veronique Paquis-Flucklinger[a],*

[a] *Université Côte d'Azur, CHU, Inserm, CNRS, IRCAN, France*
[b] *UMR7592 CNRS, Institut Jacques Monod, Université Paris Diderot, Nice, France*
[c] *Department of Inborn Errors of Metabolism and Neonatal Screening, Center of Biology and Pathology Est CHU, Lyon Bron, France*
[d] *Department of Neuropediatrics, Timone Hospital, CHU, Marseille, France*

ABSTRACT

Among mitochondrial diseases, isolated complex V (CV) deficiency represents a rare cause of respiratory chain (RC) dysfunction. In mammalian mitochondrial DNA (mtDNA), *MT-ATP6* partly overlaps with *MT-ATP8* making double mutations possible, yet extremely rarely reported principally in patients with cardiomyopathy. Here, we report a novel m.8561 C > T substitution in the overlapping region of *MT-ATP6* and *MT-ATP8* in a child with early-onset ataxia, psychomotor delay and microcephaly, enlarging the clinical manifestations spectrum associated with CV deficiency.

1. Introduction

The mitochondrial oxidative phosphorylation (OXPHOS) system, involved in cellular adenosine triphosphate (ATP) production, is composed of five complexes (complexes I-V). ATP synthase or complex V (CV) synthesizes ATP from adenosine diphosphate (ADP) in the mitochondrial matrix using the energy provided by the proton electrochemical gradient. Among mitochondrial diseases, isolated CV deficiency represents a rare cause of respiratory chain (RC) dysfunction [1,2]. The majority of CV mutations described to date is located in *MT-ATP6* and causes different clinical phenotypes including NARP (Neuropathy, Ataxia, Retinitis Pigmentosa) and Leigh syndromes (www.mitomap.org). Mutations in *MT-ATP8* have been reported more rarely [3]. Last, the 5′ part of MT-ATP6 partly overlaps with MT-ATP8 in human mitochondrial DNA (mtDNA) making double mutations possible that have been reported in a very few number of patients with cardiomyopathy [4,5]. In 2016, Laura Kytövuori et al. described a novel heteroplasmic mutation m.8561C > G in the overlapping region of *MT-ATP6* and *MT-ATP8* in two adults siblings presenting with cerebellar ataxia, peripheral neuropathy, diabetes mellitus and hypergonadotropic hypogonadism [6]. Here, we report a novel C > T substitution at

the same position (m.8561) in a child with early-onset severe neurological signs.

2. Methods

All biological samples were obtained after the obtention of informed consents. The biopsies were taken from the quadriceps femoris muscle. Spectrophotometric analysis of the individual RC complexes in muscle was performed at 37° on crude homogenates as previously described [7], and proteins were measured by Bradford microassay [8]. For Blue Native-PolyAcrylamide Gel Electrophoresis (BN-PAGE) analysis in muscle, equal amounts (15 μg) of mitochondrial proteins were subjected to blue native-PAGE, blotted onto a PVDF membrane and then incubated with specific antibodies as previously described [9]. Next-generation sequencing (NGS) of mtDNA was performed as previously described [10]. The relative proportion of the m.8561C > T was determined in different tissues by PCR-RFLP analysis. The forward primer was 5′-atg gcc cac cat aat tac cc-3′ and the reverse primer containing mismatches was 5′-cgg gta ggc cta gga t**ca** tg-3′ (mismatches shown in bold). These mismatches create a *Bsp*HI restriction site in the mutant PCR product which cuts the 193 bp amplicon into two fragments of

20 bp and 173 bp. Digested and undigested PCR products were separated through a 8% acrylamide gel. In order to analyze the conservation of mutated amino acids, we used the Web interface for EMBOSS Stretcher (https://www.ebi.ac.uk/Tools/psa/emboss_stretcher/) to calculate an optimal global alignment of human and *S. cerevisiae MT-ATP6* and *MT-ATP8* sequences. In order to predict putative impact of these substitutions on ATP synthase structure and function, we looked at their positioning on the crystallographic structure of *S. cerevisiae* mitochondrial ATP synthase [11,12] using the interface Swiss-Pdb Viewer (https://spdbv.vital-it.ch/, PDB code: 6B8H).

3. Results

3.1. Case report

The patient is a boy, born of non-consanguinous parents with no family history. He has a healthy younger sister. He presented hypotonia and microcephaly (-2DS) since age 4 months. He started associating words at age 2 years, but with dysarthria needing therapy. Walking was acquired at age 29 months and became stable only by age 6 years. Property was also acquired at 6 years. He gradually developed ataxia. Today, at age 13 years he presents ataxia increasing with effort, dysarthria, gait disorders, slowness, learning difficulties, exercise intolerance and fatigue needing to rest. Ophthalmological examination shows bilateral retinal hypoplasia. Cardiac, pulmonary and abdominal examinations are normal. Brain MRI showed bilaterally symmetric lesions in the basal ganglia, that correspond to a typical aspect of Leigh syndrome (data not shown).

Blood lactate/pyruvate ratio was slightly elevated (17; normal < 10). Histological examination of muscle was normal. Spectrophotometric analysis of the individual RC complexes in muscle revealed an isolated decrease of the CV specific activity (Supplementary Table 1). BN-PAGE analysis in patient's muscle revealed an isolated CV defect with a pattern similar to the one observed in other patients with *MT-ATP6* or *MT-ATP8* mutations (Fig. 1a) [3,6,13–15]. Amounts of the fully assembled ATP synthase were similar between patient and control. However, as previously described by Kytövuori et al., an increased amount of subcomplex F1 was observed in patient muscle with an additional assembly intermediate of complex V, referred as V*, detected in patient but not in control sample. By mtDNA NGS analysis, we identified a novel heteroplasmic substitution (m.8561C > T) in the overlapping region of *MT-ATP6* and *MT-ATP8* (Fig. 1b). This variant leads to a p.Pro12Leu substitution in *MT-ATP6* depending subunit a and, a p.Pro66Ser substitution in *MT-ATP8* depending subunit A6L of CV.
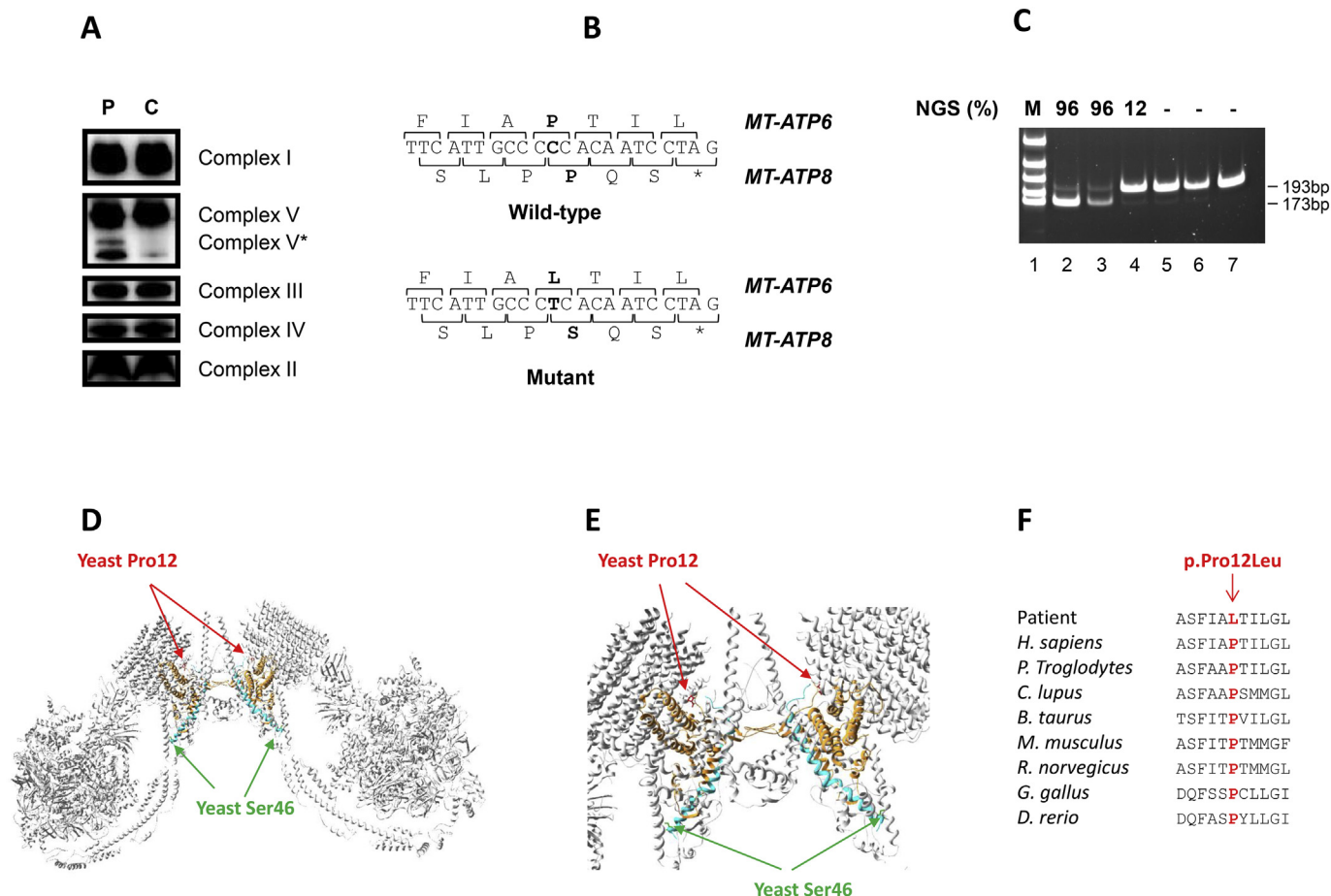

Fig. 1. A novel m.8561C > T variant associated with early-onset neurological phenotype. A. BN-PAGE in muscles of control subject (C) and patient (P). The amount of subcomplex F1 is increased in the patient and V* corresponds to the supplementary band detected by the anti-complex V antibody. (C) is matched for age and gender to the patient. B: WT and mutated mtDNA sequences obtained by NGS. C: PCR-RFLP analysis of m.8561C > T in different tissues from the patient (lane 2: muscle, lane 3 blood), his mother (lane 4: urinary epithelial cells, lane 5: buccal swab, lane 6: blood) and a negative control (lane 7). M: molecular weight marker. Mutant loads detected by NGS are indicated (top): 96% in patient's muscle and blood, 12% in patient's asymptomatic mother urines and *approximatelly the same load in her blood and buccal swab*. D–E: Localization of the concerned amino acids on the *crystallographic structure* of S. cerevisiae mitochondrial ATP synthase (e: higher magnification). The *MT-ATP6* and *MT-ATP8* subunits are in orange and blue, respectively. The Pro12 (in human and *S.cerevisiae*) is highlighted in red, and the yeast Ser46 (Pro66 in human) is in green. F: Cross-species protein conservation of *MT-ATP6*, flanking the altered proline amino acid. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

PCR-RFLP analysis showed a high mutant load (96%) in patient's muscle and blood. The patient's mother was asymptomatic. Mutant load was of 12% in urines with comparable values in blood and buccal swab (Fig. 1c).

The human *MT-ATP6* protein and the corresponding yeast protein have 31.7% identity and 51% amino acid similarity and Pro12 in human corresponds to Pro12 in yeast, indicating the conservation of this amino acid between these two organisms. With regard to the alignment of the human *MT-ATP8* and *S. cerevisiae* sequences, apart from the first four N-terminal amino acids, the sequences are poorly conserved (19.1% identity and 32.4% similarity). In addition, the amino acid sequence in yeast is shorter (20 amino acids less than in humans). According to the alignment of these two sequences, Pro66 in humans is not preserved in yeast and would correspond to Ser46.

The Pro12 (corresponding to Pro12 in humans) is located on the N-terminal loop of the *MT-ATP6* subunit and probably has a structural role in ensuring the proper positioning of the N-terminal end for the stabilization of this region of the ATPase complex. The Ser46 (corresponding to Pro66 in humans) is located at the end of an helix (Fig. 1d–e). The human Pro12 is also highly conserved through several other species which suggests a deleterious effect of the mutant (Fig. 1f).

4. Discussion

The m.8561C > G mutation, reported by Kytövuori's et al., leads to p.Pro12Arg substitution in *MT-ATP6* predicted to be pathogenic and p.Pro66Ala substitution in *MT-ATP8* predicted to be neutral [6]. Muscle heteroplasmy was very high (99%) with decreased ATP production and impaired assembly of complex V similar to what we found in our patient's muscle. Patients described by Kytövuori's et al. and the one we report have an ataxia. The phenotype is more severe in our case with psychomotor delay and microcephaly appearing during first months of life. However, we have no objective argument to explain this phenotypic variability, frequently found in mitochondrial diseases.

5. Conclusion

In conclusion, our results confirm the importance of proline residues at position 12/66 in *MT-ATP6/8* and enlarge the spectrum of clinical manifestations associated with CV deficiency.

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Author's contributions

KF performed the biochemical examinations, analyzed and interpreted of the patient's data and wrote the manuscript with VP-F supervision. CA, SB and CR performed the molecular examinations, analyzed and interpreted of the patient's data. AC and BC expertized clinical data. VS performed the crystallographic studies. All authors have been involved in drafting the manuscript or revising it critically for important intellectual content, given final approval of the version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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