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A novel m.12908T>A mutation in the mitochondrial *ND5* gene in patient with infantile-onset Pompe disease

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ABSTRACT

Pompe disease is a progressive metabolic myopathy caused by deficiency in lysosomal acid α-glucosidase and results in cellular lysosomal and cytoplasmic glycogen accumulation. A wide spectrum of clinical phenotypes exists from hypotonia and severe cardiac hypertrophy in the first few months of life to a milder form with the onset of symptoms in adulthood. The disease is typically due to severe mutations in *GAA* gene. In the present study, we described a newborn boy with clinical features of Pompe disease particularly with hypertrophic cardiomyopathy, hypotonia and hepatomegaly. This case was at first misdiagnosed as mitochondrial disorder. Accordingly, we performed a mitochondrial mutational analysis that revealed a novel mutation m.12908T>A in the *ND5* gene. Secondary structure analysis of the ND5 protein further supported the deleterious role of the m.12908T>A mutation, as it was found to involve an extended imbalance in its hydrophobicity and affect its function.

1. Introduction

Pompe disease (glycogen storage disease type II, acid maltase deficiency, OMIM #232300) is a rare autosomal recessive neuromuscular disorder, in which a deficiency of acid α-glucosidase leads to a progressive intralysosomal accumulation of glycogen in various tissues, among which skeletal and cardiac muscle are the most important [1].

The disease is often severe and occurs with a wide spectrum of clinical phenotypes, varying by age of onset, level of organ involvement, and degree of myopathy. This disorder is characterized by a hypertrophic cardiomyopathy, a hypotonia, amacroglossia, and a swallow difficulty. The infantile form of the disease is fatal, in absence of treatment, with death from cardiorespiratory failure or respiratory infection occurring usually within the first 1–2 years of life [2]. The later-onset form of the disease is more slowly progressive, more heterogeneous and typically presents without cardiac manifestations [3,4].

It was usually considered that Pompe disease is caused only by severe mutations in the gene-encoding acid α-glucosidase (*GAA*). Both copies of the *GAA* gene need to harbor a pathogenic mutation before the disease manifests itself as partial or complete loss of GAA activity [2,5]. However, the clinical diversity observed within a large group of patients with functionally the same GAA genotype demonstrates that modifying factors can have a substantial effect on the clinical course of Pompe disease, disturbing the GAA genotype–phenotype correlation [6,17]. The similarity of phenotypes observed in Pompe disease and in several mitochondrial cytopathies suggests the implication of mitochondrial DNA in patient with Pompe disease. In fact, mitochondrial mutations could be detected in several autosomal recessive diseases such as Wolfram syndrome [8–11].

In this study, we reported a novel m.12908T>A mitochondrial mutation (p.191L>Q) in the NADH–dehydrogenase subunit 5 (*ND5*) gene, in patient with clinical features of Pompe disease particularly with a hypertrophic cardiomyopathy, a hypotonia and a hepatomegaly.

2. Patients and methods

2.1. Patients

The patient was the third child of healthy consanguineous Tunisian parents. He was born at term after an uncomplicated pregnancy and delivery with birth weight of 2.80 kg, height 48 cm and head circumference 33 cm. He had one brother and one sister who are healthy.

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Table 1
Punctual mitochondrial variations detected in the coding region in the studied patient and the phenotypic association.

Locus	Nucleotide change	Aminoacid change	Phenotypic association
MT-RNR1	m.742T>C	Non coding	Hearing loss
MT-RNR1	m.750A>G	Consensus	Auditory neuropathy; CPEO; Hearing loss; Hypertension ; **Hypertrophic cardiomyopathy**; Idiopathic Bilateral vestibulopathy; **Infantile cardiomyopathy**; **Left ventricular noncompaction**; Leigh syndrome; LHON; MELAS; MERRF; Mitochondrial encephalomyopathy; Muscle disease; Respiratory dysfunction; Type 2 diabetes mellitus ;
MT-RNR1	m.769G>A	Non coding	Hearing loss; Idiopathic bilateral vestibulopathy; **Hypertrophic cardiomyopathy**; **Left ventricular noncompaction**; LHON
MT-RNR1	m.1018G>A	Non coding	Colorectal cancer; Hearing loss; **Hypertrophic cardiomyopathy**; Idiopathic bilateral vestibulopathy; **Left ventricular noncompaction**
MT-RNR2	m.2626T>C	Non coding	Muscle pathology; MELAS; Mitochondrial encephalomyopathy
MT-RNR2	m.2706A>G	Non coding	Muscle pathology; Auditory neuropathy; Diabetes; Hearing loss; Hypertension; **Hypertrophic cardiomyopathy**; **Idiopathic cardiomyopathy**; **Infantile cardiomyopathy**; Leigh syndrome; LHON; MELAS; MERRF; Mitochondrial encephalomyopathy; Mitochondrial myopathy; Muscle disease
MT-ND1	**m.3321C>T**	**Syn**	
MT-ND1	m.3594C>T	Syn	LHON; Parkinson disease; Prostate cancer
MT-ND1	m.3654C>T	Syn	-
MT-ND1	m.3849G>A	Syn	Hearing loss ; Parkinson disease
MT-ND1	m.4104A>G	Syn	Colorectal cancer; **Dilated cardiomyopathy**; Hearing loss; **Hypertrophic cardiomyopathy**; Parkinson disease
MT-ND1	m.4185C>T	Syn	LHON
MT- COI	m.6040A>C	p.46N>T	-
MT- COI	m.6497T>C	Syn	-
MT-TD	m.7521G>A	Non coding	Encephalopathy; Hearing loss; **Left ventricular noncompaction**
MT-CO2	m.8206G>A	Syn	Hearing loss; **Hypertrophic cardiomyopathy**; **Mitochondrial cardiomyopathy**
MT-CO2	m.8701A>G	p.59 T>A	Auditory neuropathy; Hearing loss; Hypertension; **Hypertrophic cardiomyopathy**; **Infantile cardiomyopathy**; LHON; MELAS; Mitochondrial encephalomyopathy; Mitochondrial diabetes; **Myocardial infarction**; Leigh syndrome

At the age of 4 months, he was hospitalized since he showed a global hypotonia and hepatic cytolysis. An abdominal exploration suggests a hepatomegaly. Neurological examination showed an axial and peripheral hypotonia. In addition, his blood pressure was 96/63 mmHg, with heart rate of 80 bpm and cardiology consultation showed the absence of mitral murmur. Besides, thorax radiography showed a cardiothoracic index of 0.64 and a spherical appearance of the heart suggesting a cardiomegaly. The electrocardiogram demonstrated a cardiac frequency of 140 bpm and a wide QRS complex indicating ventricular impairment.

The above clinical findings were first highly suggestive of a mitochondrial disease and the patient was further investigated.

Laboratory explorations revealed a normal creatine kinase level (65.3 units/l) (reference range: 60–305 units/l) and a normal lactate level (0.94 mmol/l) (reference range: 0.5–1.8 mmol/l). His complete blood count (CBC) was: white blood cell (WBC): 7410 mm^{-3}, hemoglobin (Hb): 12.1 g/dl, Platelet count: 412,000 mm^{-3}. A liver failure was demonstrated with the presence of elevated levels of transaminases (AST: 74 UI/l), alkaline phosphatase (353 UI/l) and also of gamma glutamyl transferase (60 UI/l).

The diagnosis of Pompe disease was confirmed after a molecular testing which revealed a homozygous mutation of the *GAA* gene. However, the patient was untreated and he died at the age of 3 years suffering from severe respiratory distress.

In addition, 100 Tunisian healthy individuals were tested as controls. These controls should have no personal or family history of any disorder. All individuals (patients and controls) provided informed consent.

Table 1 (*continued*)

MT-CO2	m.8860A>G	p.112T>A	Muscle pathology; Auditory neuropathy; Early-onset dystonia; Hearing loss; Hypertension; **Hypertrophic cardiomyopathy** **Idiopathic cardiomyopathy**; **Infantile cardiomyopathy**; Leigh syndrome; LHON; MELAS; MERRF ; Mitochondrial encephalomyopathy; Respiratory dysfunction; Type 2 diabetes mellitus
MT-CO3	m.9540T>C	Syn	Alzheimer's disease; Auditory neuropathy; Diabetes; Hearing loss; Hypertension; **Hypertrophic cardiomyopathy**; **Infantile cardiomyopathy**; LHON; MELAS; Mitochondrial encephalomyopathy; **Myocardial infarction**
MT-CO3	m.11719G>A	Syn	Auditory neuropathy; **Cardiomyopathy**; Complex I deficiency; Hearing loss; Hypertension; **Hpertrophic cardiomyopathy**; **Infantile cardiomyopathy**; Lactic acidosis; Leigh syndrome; LHON ; MELAS; MERRF; Mitochondrial encephalomyopathy; Mitochondrial myopathy; Parkinson disease; Respiratory dysfunction
MT-CO3	m.11944 T>C	Syn	Hearing loss; **Idiopathic cardiomyopathy**; LHON; Parkinson's disease
MT-TL2	m.12311T>C	Syn	Bipolar disorder ; Lactic acidosis
MT-ND5	m.12630G>A	Syn	Colorectal cancer ; Hearing loss; Mitochondrial encephalomyopathy
MT-ND5	m.12705 C>T	Syn	Alzheimer's disease; Auditory neuropathy; Colorectal cancer; Diabetes; Hearing loss; Hypertension; **Idiopathic cardiomyopathy**; **Infantile cardiomyopathy**; Leigh syndrome; LHON; MELAS; MERRF; Metabolic disease; Mitochondrial encephalomyopathy; **Myocardial infarction**; Respiratory dysfunction ;
MT-ND5	m.12879 T>C	Syn	Hearing loss; Mitochondrial encephalomyopathy
MT-ND5	**m.12908 T>A**	**p.191L>Q**	
MT-CYB	m.14890A>G	Syn	-
MT-CYB	m.15301G>A	Syn	Auditory neuropathy; Hearing loss; Hypertension; LHON; MELAS; Mitochondrial encephalomyopathy; **Myocardial infarction**; Parkinson disease
MT-CYB	m.15326A>G	p.194T>A	Auditory neuropathy; Complex III deficiency; Exercise intolerance; Hearing loss; **Histiocytoid cardiomyopathy**; Hypertension; **Hypertrophic cardiomyopathy**; **Idiopathic cardiomyopathy**; **Infantile cardiomyopathy**; Leigh syndrome; LHON; MELAS; MERRF; Mitochondrial encephalomyopathy ; Non-syndromic deafness
MT-CYB	m.15451C>G	p.235F>L	Hearing loss

LHON: Leber hereditary optic neuropathy; MELAS: mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MERRF: myoclonus epilepsy associated with ragged-red fibers; CPEO: chronic progressive external ophthalmoplegia; COX: cytochrome c oxidase.
Novel variations are highlighted and written in bold.

2.2. Methods

2.2.1. DNA extraction

Total genomic DNA was extracted using phenol–chloroform standard procedures from blood samples of the proband and her mother following informed consent [12].

2.2.2. Mutational analysis of the mitochondrial genes

The entire mitochondrial DNA was amplified using 24 overlapping pairs of primers in a thermal cycler GeneAmp PCR System 2720 (Applied Biosystems). Each 50 μl PCR reaction contains 2 μg of total DNA, 8 pmol of each primer, 2 mM $MgCl_2$, 500 μM dNTP, 1× PCR buffer and 2U Taq DNA polymerase. The program starts with an initial denaturation followed by to 35 cycles (94 °C for 1 min, 56.5 °C for 1 min and 72 °C for 1 min) and a final elongation at 72 °C.

PCR products were purified using NucleoSpin (MACHEREY–NAGEL) and sequenced in an ABI PRISM 3100-Avant automated DNA sequencer using the BigDye Terminator Cycle Sequencing reaction kit v1.1. The blast homology searches were performed using the programs available at the National Center for Biotechnology Information Website compared to the revised Cambridge Reference Sequence (rCRS) [13].

Regions containing putative novel variations were amplified and sequenced again on both strands to exclude that they were PCR artefacts.

2.2.3. RFLP–PCR analysis for quantification of the proportion of the mutant mitochondrial DNA

The presence of the m.12908T>A point mutation was confirmed by using mismatch PCR reactions in which a restriction site for the endonuclease *BfaI* was created in the presence of the mutation. The oligonucleotide primers used for the *BfaI* mismatch PCR were: AAACAACCCAGCTCTCCCTAA (12551–12571) for the forward primer and TGGGTCTCATGAGTTGGAGT**C**T (12930–12909) for the reverse one (mismatch nucleotide is underlined and written in bold).

Table 2
Punctual mitochondrial variations detected in the non-coding region in the studied patient and the phenotypic association.

Locus	Nucleotide change	Phenotypic association
MT-D-Loop (MT-HV2)	m.73A>G	Alzheimer's Disease; Diabetes; Hearing loss; Hypertension; **Hypertrophic cardiomyopathy**; **Infantile cardiomyopathy**; Leigh syndrome; LHON; MELAS; Mitochondrial encephalomyopathy ; Nasopharyngeal carcinoma; Nonsyndromic deafness; Optic neuropathy; Parkinson's disease
MT-D-Loop (MT-HV2)	m.146T>C	Auditory neuropathy; Esophageal carcinoma; Friedreich's ataxia; Hearing loss; LHON; Mitochondrial encephalomyopathy; Muscle disease; Ovarian cancer
MT-D-Loop (MT-HV2)	m.152T>C	Alzheimer's disease; Diabetes; Friedreich's ataxia; Hearing loss; LHON; Mitochondrial encephalomyopathy; Nasopharyngeal carcinoma; Ovarian cancer
MT-D-Loop (MT-HV2)	m.182C>T	LHON ; Low sperm motility
MT-D-Loop (MT-HV2)	m.183A>G	Leukemia; Nasopharyngeal carcinoma; Mitochondrial myopathy; Ovarian carcinomas
MT-D-Loop (MT-HV2)	m.186C>T	Hearing loss
MT-D-Loop (MT-HV2)	m.263A>G	Auditory neuropathy; Diabetes; Early-onset dystonia; Friedreich's ataxia; Hearing loss; Hypertension; **Hypertrophic cardiomyopathy**; **Infantile cardiomyopathy**; Klinefelter's syndrome; Leigh syndrome; LHON; MELAS; MERRF; Mitochondrial encephalomyopathy; Mitochondrial myopathies; Nasopharyngeal carcinoma
MT-D-Loop (MT-HV1)	m.16212A>G	Alzheimer's disease; Parkinson's disease
MT-D-Loop (MT-HV1)	M.16223C>T	Alzheimer's disease; Muscle pathology; Auditory neuropathy; Complex I deficiency; Diabetes; Hearing loss; Hypertension; **Hypertrophic Cardiomyopathy**; **Idiopathic cardiomyopathy**; **Infantile cardiomyopathy**; Leigh syndrome; Leukemia; LHON; Low sperm motility; Mitochondrial encephalomyopathy; Mitochondrial myopathies; Nasopharyngeal carcinoma; Ovarian cancer
MT-D-Loop (MT-HV1)	M.16224T>C	**Hypertrophic Cardiomyopathy**; Leukemia; LHON; Occipital stroke; Ovarian cancer; Parkinson's disease; Reduced spermatozoa motility
MT-D-Loop (MT-HV1)	m.16278C>T	Hearing loss; Hypertension; **Idiopathic cardiomyopathy**; Leigh syndrome; Leukemia; LHON; Low sperm motility; Mitochondrial encephalomyopathy; Mitochondrial myopathies; Metabolic syndrome
MT-D-Loop (MT-HV1)	m.16309A>G	Hearing loss; Leukemia; Nasopharyngeal carcinoma; Occipital stroke
MT-D-Loop (MT-HV1)	m.16390G>A	Alzheimer's disease; Friedreich's ataxia; Hearing loss; Leukemia; LHON; Nasopharyngeal carcinoma; Sudden infant death syndrome
MT-D-Loop (MT-HV1)	m.16399A>G	**Infantile cardiomyopathy**; Hearing loss; LHON; Mitochondrial encephalomyopathy; Occipital stroke; Ovarian cancer; Sudden infant death syndrome
MT-D-Loop (MT-HV1)	m.16519T>C	Alzheimer's disease; Muscle pathology; Diabetes; **Dilated cardiomyopathy**; Hearing loss; Hypertension; **Idiopathic cardiomyopathy**; **Infantile cardiomyopathy**; Leukemia; Leigh syndrome; LHON; MELAS; MERRF; Metabolic syndrome; **Mitochondrial cardiomyopathy**; Mitochondrial encephalomyopathy; Nasopharyngeal carcinoma; Occipital stroke; Ovarian cancer

LHON: Leber hereditary optic neuropathy; MELAS: mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MERRF: myoclonus epilepsy associated with ragged-red fibers; CPEO: chronic progressive external ophthalmoplegia; COX: cytochrome c oxidase.
Novel variations are highlighted and written in bold.

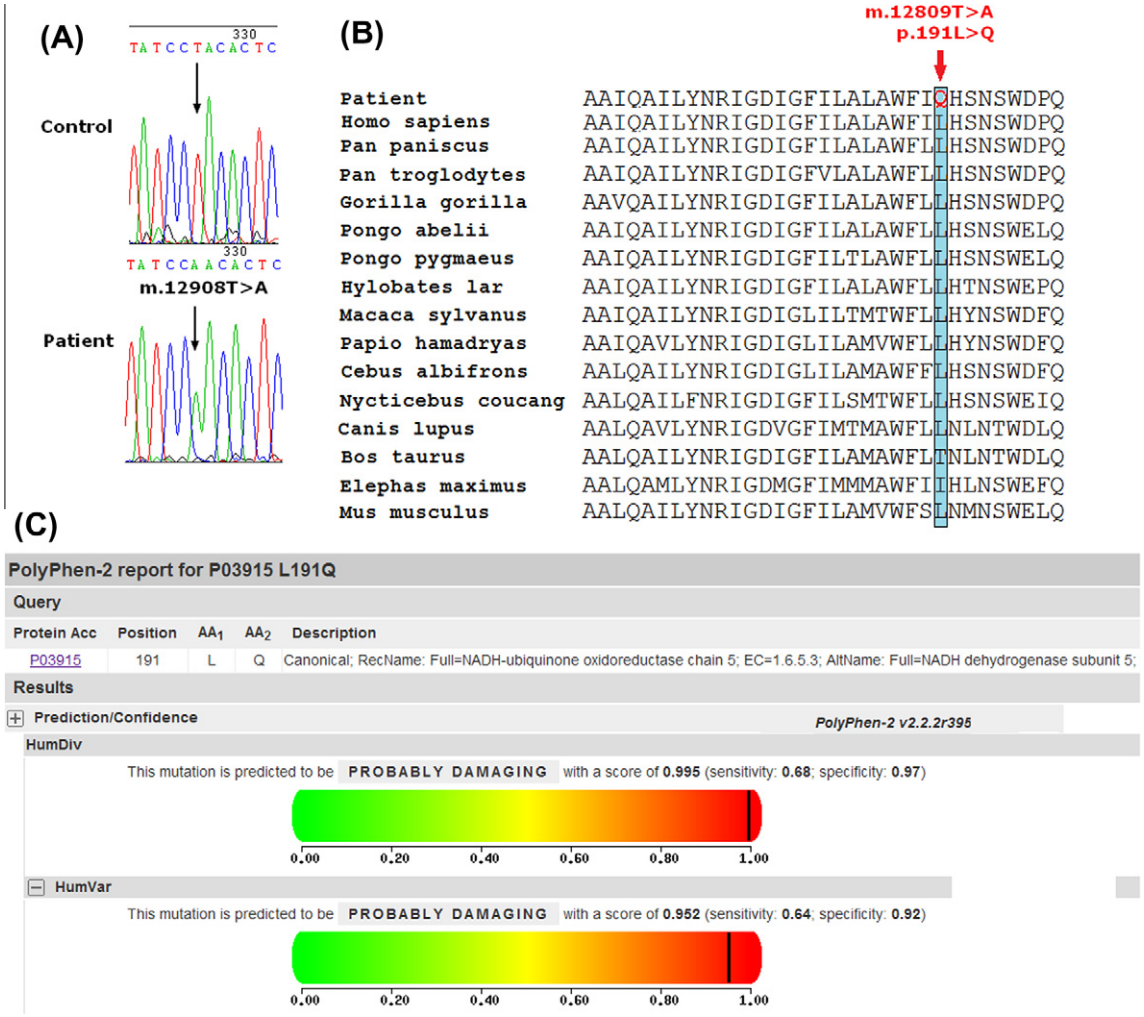

Fig. 1. (A) Sequence chromatograms showing the presence of the m.12809T>A mutation in the mitochondrial *ND5* gene in the studied patient and its absence in a control subject. (B) Global alignment of the amino acid sequences of ND5 protein belonging to 20 different eukaryotic organisms. Amino acid change of interest is framed and indicated with arrow. (C) Results of the PolyPhen-2 analysis predicting the pathogenicity the p.191L>Q substitution on ND5 protein.

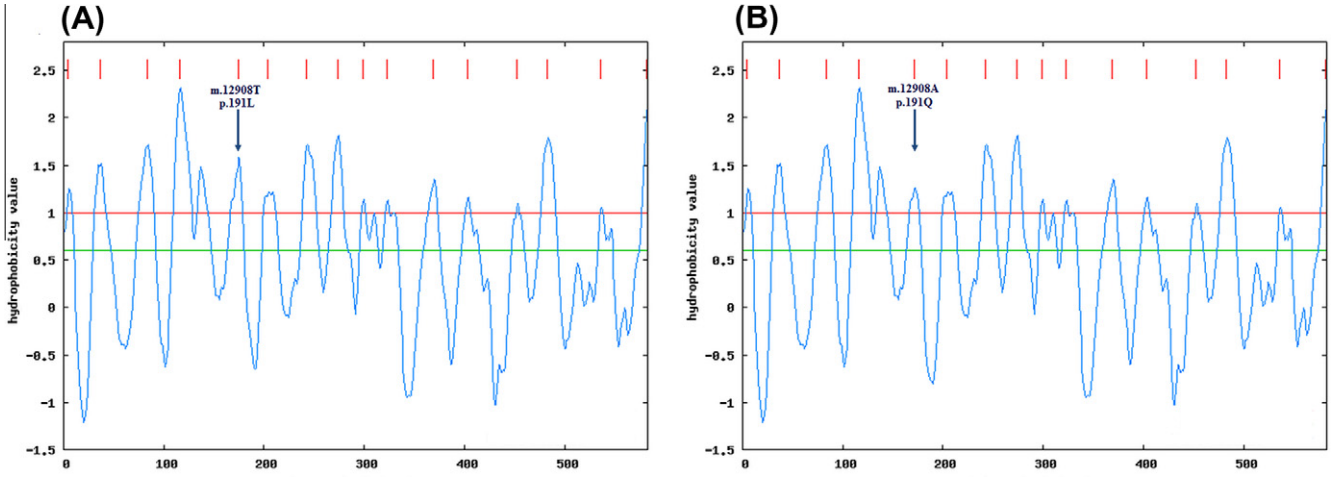

Fig. 2. A hydropathy plot for the ND5 subunit. The hydrophobicity of the wild-type ND5 subunit (A) is compared to the mutant form, including novel m.12908T>A mutation (B), mutated site shown by arrow.

In the presence of the wild-type nucleotide at position 12908, the 328 bp PCR product is cleaved into 2 fragments of 305 and 23 bp. PCR was performed in 35 cycles of denaturation (94 °C, 30 s), annealing (50 °C, 30 s), and extension (72 °C, 40 s).

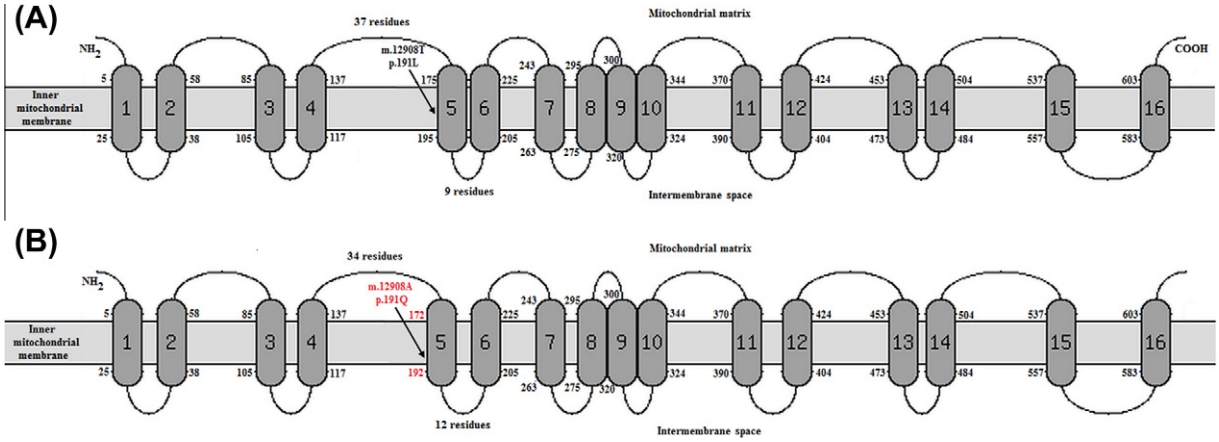

Fig. 3. Predicted transmembrane structures of human wild-type MT-ND5 protien (A) and in presence of the novel m.12908T>A mutation (B) by the TopPred program. This substitution substantially reduced the hydrophobicity of the intramembrane helical domain, but also influenced both contiguous matrix and intermembrane space coils.

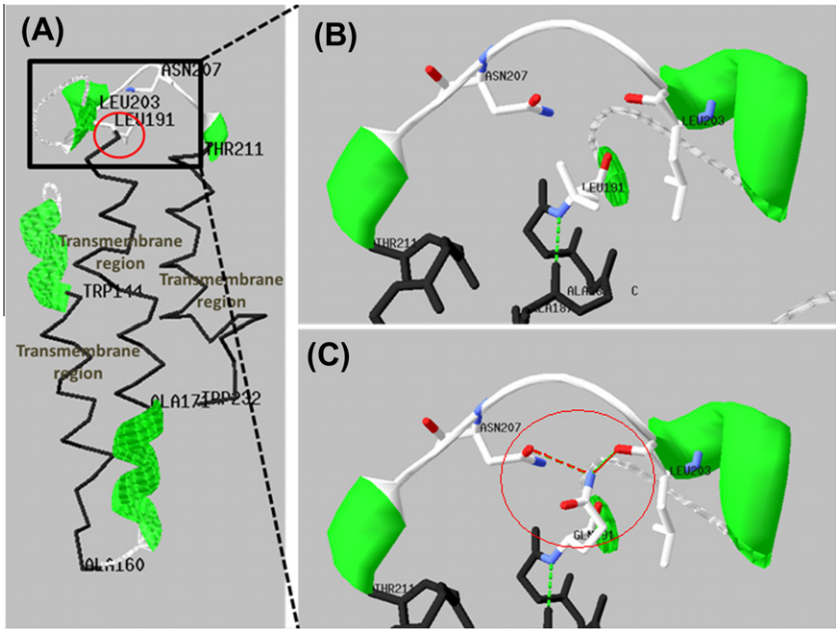

Fig. 4. Model generation by molecular modeling by homology of MT-ND5. (A) Model of 133–233 region of the wild MT-ND5 protein (191L). (B) Hydrogen bound between L191 and A187 residues. (C) Hydrogen bound between Q191, N207 and L203 residues.

2.2.4. The pathogenicity prediction of the m.12908T>A and prediction software of hydrophobicity and secondary structure of the MT-ND5 protein

The assessment of the damaging effect of missense mutation was performed using PolyPhen-2 software (http://genetics.bwh.harvard.edu/pph2/). This software calculates the probability that a given mutation is damaging and reports estimates of false positive (the chance that the mutation is classified as damaging when it is in fact non damaging) and true positive (the chance that the mutation is classified as damaging when it is indeed damaging) rates. For a false positive rate of 20%, PolyPhen-2 achieved true positive prediction rates of 92% and 73% on HumDiv and HumVar datasets, respectively [14].

For the prediction of possible changes in hydrophobicity and changes in configuration of transmembrane domains of the ND5 protein due to sequence variation, we used TopPred online prediction software (http://bioweb.pasteur.fr/seqanal/interfaces/toppred.html).

2.2.5. Generation of a 3D model of MT-ND5

In order to investigate the eventual effect of the non synonymous variation changing a leucine to a glutamine at position p.191 (p.191L>Q) of MT-ND5 protein, we modeled and compared the two variants, 191L and 191Q.

We used PSI-BLAST to select the best template "3RKO" PDB structure, which has a homology of 39% with the spanned A134 and W232 of MT-ND5 protein sequence [15]. 3RKO is a structure of NADH–quinone oxidoreductase of *Escherichia coli*. The Clustal W algorithm was used to optimize alignments of the target and template protein sequences [16]. The generation of the two theoretical 3D models was achieved by MODELLER9v8 [17] software. The SWISS PDB VIEWER software (V3.7) was used to display and compare models. The quality of the models was evaluated using ProSA-web [18]. No difference was found between the two models. Once the two models superimposed, the RMS calculated between backbone atoms was low (0.33 Å). For the two models, the Rama-

chandran plot showed that more than 90%, of atoms were in permitted areas. Thus we concluded that they were correct.

2.2.6. Mitochondrial haplogroup analysis and sequence alignment

Haplogroups are defined by nucleotides at specific known polymorphic sites in the mtDNA. After sequencing the entire mitochondrial DNA, we performed mitochondrial haplogrouping analysis using MitoTool database (http://www.mitotool.org/genome.html) [19].

3. Results

In this study, we report a 4-months-old patient with clinical features of Pompe disease associated with hypertrophic cardiomyopathy.

The analysis of the mtDNA extracted from the blood leucocytes in the studied patient revealed two novel variations. In fact, we found a novel m.12908T>A transversion in the mitochondrial *ND5* gene. It was a missense mutation which was absent in 100 healthy controls from Tunisian population. We also detected a novel m.3321C>T transition in the mitochondrial *ND1* gene which was a silent variation and was considered as a polymorphism.

Moreover, we detected 28 known substitutions (Table 1) and 15 variations in the D-loop region (Table 2) which were previously reported in the Human Mitochondrial Database (http://www.mitomap.org). Among these variations, 5 substitutions were responsible for an amino acids change in several mitochondrial subunits: m.6040A>C (p.46N>T in MT-CO1), m.8701A>G (p.59T>A in MT-CO2), m.8860A>G (p.112T>A in MT-CO2), m.15326A>G (p.194T>A in MT-CYB) and m.15451C>G (p.235F>L in MT-CYB). These substitutions were reported as polymorphisms without negative effects on mitochondrial function. We also found 4 novel mitochondrial variations: m.3654C>T, m.6040A>C, m.6497T>C and m.14890A>G. In addition, we identified several variations in polymorphic sites that allowed us to classify the studied patient under the sub-haplogroup Ha2a2.

The novel transversion m.12908T>A in the mitochondrial *ND5* gene substituted the leucine residue at position 191 to a glutamine (p.191L>Q) (Fig. 1A).

The analysis of the mitochondrial ND5 polypeptide sequences from different species showed that the leucine residue was localized in an evolutionarily stable domain (Fig. 1B). In addition, PolyPhen-2 analysis predicted that this variant is probably damaging with scores of 0.995 and 0.952 on HumDiv and HumVar models, respectively (Fig. 1C). Moreover, this transversion substituted a large, non-polar, hydrophobic leucine (with hydropthy index 3.8) at aminoacid in position 191 of the ND5 subunit to a polar hydrophilic glutamine (with hydropthy index −3.5). The modeling of the protein secondary structure determined that ND5 is largely a hydrophobic protein, but it contains also a few hydrophilic loops. A hydropathy plot of the p.191L>Q mutant polypeptide generated with the Kyte–Doolittle algorithm demonstrated an extended imbalance in its hydrophobicity caused by the m.12908T>A mutation (Fig. 2). This substitution substantially reduced the hydrophobicity of the intramembrane helical domain, but also influenced both contiguous matrix and intermembrane space coils (Fig. 3).

Besides, generation of a 3D model of MT-ND5 demonstrated that the presence of amine functional group in the side chain allows to Q191 a more chance than L residue to share a hydrogen bund with N207 and L203 neighbor residues (Fig. 4). These two additional hydrogen bunds may affect the spatial conformation and the shape of this important region implicated in the cation transport.

4. Discussion

We described a Tunisian child with clinical features of infantile-onset Pompe disease including hypertrophic cardiomyopathy, global hypotonia and hepatomegaly that were mainly associated with mitochondrial disorders. Ultimately the diagnosis of Pompe disease was confirmed via the acid α-glucosidase deficiency and the presence of the c.236_246del homozygous mutation in *GAA* gene (Data not shown).

The sequencing analysis of the whole mitochondrial DNA in this patient revealed a novel m.12908T>A variation in the mitochondrial *ND5* gene, which may be highly a causal mutation given the fact that this variant was not present in the mitochondrial databanks nor in the normal population control.

The ND5 subunit is a hydrophobic subunit of complex I encoded by the mitochondrial genome. Its bacterial homologue, the NDH-1 subunit (NuoL), acts as a cation transporter in the absence of other NDH-1 subunits [20,21]. The central fragment of MT-ND5 constitutes the most conserved region, exhibiting the highest sequence similarity to cation/H1 antiporters [22].

According to the TopPred prediction, the m.12908T>A mutation causes a substitution of the hydrophobic Leu191 by glutamine (p.191L>Q) in the fifth transmembrane helix of the ND5 subunit in a border zone between transmembranaire space and mitochondrial matrix. This location is similar to the mitochondrial ND6 variants essentially the LHON mutations which are located in transmembrane helices B or C of the ND6 polypeptide [23].

The highly conservation of the amino acid at the position 191 (Fig. 1B) suggests that the replacement within the α-helix could change the tertiary structure of the ND5 subunit, and consequently the hydrophobic part of the complex I. In addition, generation of a 3D model of MT-ND5 revealed that in presence of leucine residue, two hydrogen bunds are present. Consequently, the L191Q substitution caused by our mutation may affect the function and the stability of MT-ND5 protein (Fig. 4).

The description of the m.12908T>A mutation in our patient confirms that the mitochondrial *ND5* gene is as a "hot spot" for mtDNA mutations described with clear evidence of pathogenicity [24–26]. Particularly, mutations in *ND5* gene were frequently reported in various phenotypes including MELAS, LHON, MERRF and Leigh syndrome as well as overlap mitochondriopathy syndromes [27,28]. By studying specific mutations affecting mitochondrial NADH deshydrogenase subunits, several progresses have been made in the comprehension of their role in the assembly/stability of the complex. Some mutations in *ND5* gene affect the enzymatic activity of complex I but not its assembly status [29,30]. Since ND5 is the last ND subunit to be incorporated into the membrane arm [31], it is likely not essential for the assembly of the other mtDNA NADH deshydrogenase subunits into the complex. However, the loss of ND5 is associated with instability of the membrane arm [32]. The precise contribution of the ND5 protein to complex I function is not entirely clear, although it may be important for ubiquinone binding and thus, the electron transfer within the complex. In addition, the physiological importance of the ND5 subunit has been shown by studies reporting that complex I-dependent respiration is tightly regulated by *ND5* gene expression [33], and the ND5 subunit is essential for the activity of complex I [34].

Mitochondrial structural abnormalities were described in previous studies in skeletal muscle biopsy derived from a patient with Pompe disease. These mitochondria were imperfect oval, polygonal, or prism shaped and contained dense granular material and paracrystalline inclusions located in inter-cristae space [35,36]. Dysfunctional mitochondria with swollen cristae were also ob-

served in induced pluripotent stem cells derived from the fibroblasts of two patients with Pompe disease [35]. In addition, morphological evidence of abnormal autophagy in muscle biopsies from patients with Pompe disease has been presented in several reports [36,37]. Autophagy is a major intracellular catabolic pathway that delivers long-lived proteins and damaged organelles, in particular mitochondria, to lysosomes for degradation and recycling [38,39]. Moreover, it was mentioned that suppression of autophagy in skeletal muscle in wild type mice leads to accumulation of enlarged and dysmorphic mitochondria [40].

To our knowledge, our present study describes the first mitochondrial mutation associated with infantile-onset Pompe disease. Despite the disease was usually associated to mutations in *GAA* gene, the m.12908T>A mutation could be highly implicated in the pathophysiology of Pompe disease. In fact, occurrence of modifying factors in Pompe disease has been supposed [6,7] as well as mitochondrial mutations was detected in several autosomal recessive diseases such as Wolfram syndrome [8–11]. Conduction defects such as Wolff–Parkinson–White syndrome was also reported in patients with both mtDNA and nuclear-encoded complex I defects [41].

Conflict of interest

The authors declare that they have no competing interests.

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