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The mitochondrial ND1 m.3337G>A mutation associated to multiple mitochondrial DNA deletions in a patient with Wolfram syndrome and cardiomyopathy

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ABSTRACT

Wolfram syndrome (WFS) is a rare hereditary disorder also known as DIDMOAD (diabetes insipidus, diabetes mellitus, optic atrophy, and deafness). It is a heterogeneous disease and full characterization of all clinical and biological features of this disorder is difficult. The wide spectrum of clinical expression, affecting several organs and tissues, and the similarity in phenotype between patients with Wolfram syndrome and those with certain types of respiratory chain diseases suggests mitochondrial DNA (mtDNA) involvement in Wolfram syndrome patients. We report a Tunisian patient with clinical features of moderate Wolfram syndrome including diabetes, dilated cardiomyopathy and neurological complications. The results showed the presence of the mitochondrial ND1 m.3337G>A mutation in almost homoplasmic form in 3 tested tissues of the proband (blood leukocytes, buccal mucosa and skeletal muscle). In addition, the long-range PCR amplifications revealed the presence of multiple deletions of the mitochondrial DNA extracted from the patient's skeletal muscle removing several tRNA and protein-coding genes. Our study reported a Tunisian patient with clinical features of moderate Wolfram syndrome associated with cardiomyopathy, in whom we detected the ND1 m.3337G>A mutation with mitochondrial multiple deletions.

1. Introduction

First described in 1938, Wolfram syndrome (WFS) (MIM 222300) is a rare hereditary disorder also known as DIDMOAD (diabetes insipidus, diabetes mellitus, optic atrophy, and deafness) [1,2]. This is a progressive, neurodegenerative disorder, with diabetes mellitus and optic atrophy presenting in the first decade, cranial diabetes insipidus, and sensorineural deafness in the second, and neuropathic bladder in the third, followed by neurological complications in the fourth decade [3,4].

Nevertheless, such manifestations are not always present and we can note the association with other diverse neurological and psychiatric features, including ataxia, peripheral neuropathy, dementia and depression. Thus, Wolfram syndrome seems to be a heterogeneous disease and full characterization of all clinical and biological features of this disorder is difficult because, with the exception of a few series, the number of patients in most reports is small. The pathogenesis of the disease is still unknown.

Diagnosis is usually established in one or several siblings from unaffected parents suggesting an autosomal recessive mode of transmission [5]. In fact, one candidate gene WFS1 (wolframin) has been mapped to chromosome 4p and was recently cloned and localized [6–8]. It was shown to encode a transmembrane protein called wolframin whose function is not yet determined.

Nevertheless, the wide spectrum of clinical expression, affecting several organs and tissues, and the similarity in phenotype between patients with Wolfram syndrome and those with certain types of respiratory chain diseases suggests mitochondrial DNA (mtDNA) involvement in Wolfram syndrome patients. This hypothesis was confirmed in sporadic cases that showed a loss of mitochondrial function and the presence of heteroplasmic deletion in mtDNA [9–11]. In addition, authors suggested that mtDNA variants (4216 and 11,251) may predispose to Wolfram syndrome [12].

In the present study, we report a Tunisian patient with clinical features of moderate Wolfram syndrome including diabetes, dilated cardiomyopathy and neurological complications. His brother was died from a typical Wolfram syndrome since he suffered from diabetes insipidus, diabetes mellitus, optic atrophy, and

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Table 1
Primers used for the long-range PCR amplifications.

Size	Primer	Sequences	Nucleotidic positions
4068 bp	mito18F	5′-TATCACTCTCCTACTTACAG-3′	11,929–11,948
	mito22R	5′-AGCTTTGGGTGCTAATGGTG-3′	15,997–15,978
6786 bp	mito14F	5′-CCCACCAATCACATGCCTAT-3′	9211–9230
	mito22R	5′-AGCTTTGGGTGCTAATGGTG-3′	15,997–15,978
8089 bp	mito12F	5′-ACGAGTACACCGACTACGGC-3′	7908–7927
	mito22R	5′-AGCTTTGGGTGCTAATGGTG-3′	15,997–15,978
10,162 bp	mito9F	5′-GAGGCCTAACCCCTGTCTTT-3′	5835–5854
	mito22R	5′-AGCTTTGGGTGCTAATGGTG-3′	15,997–15,978

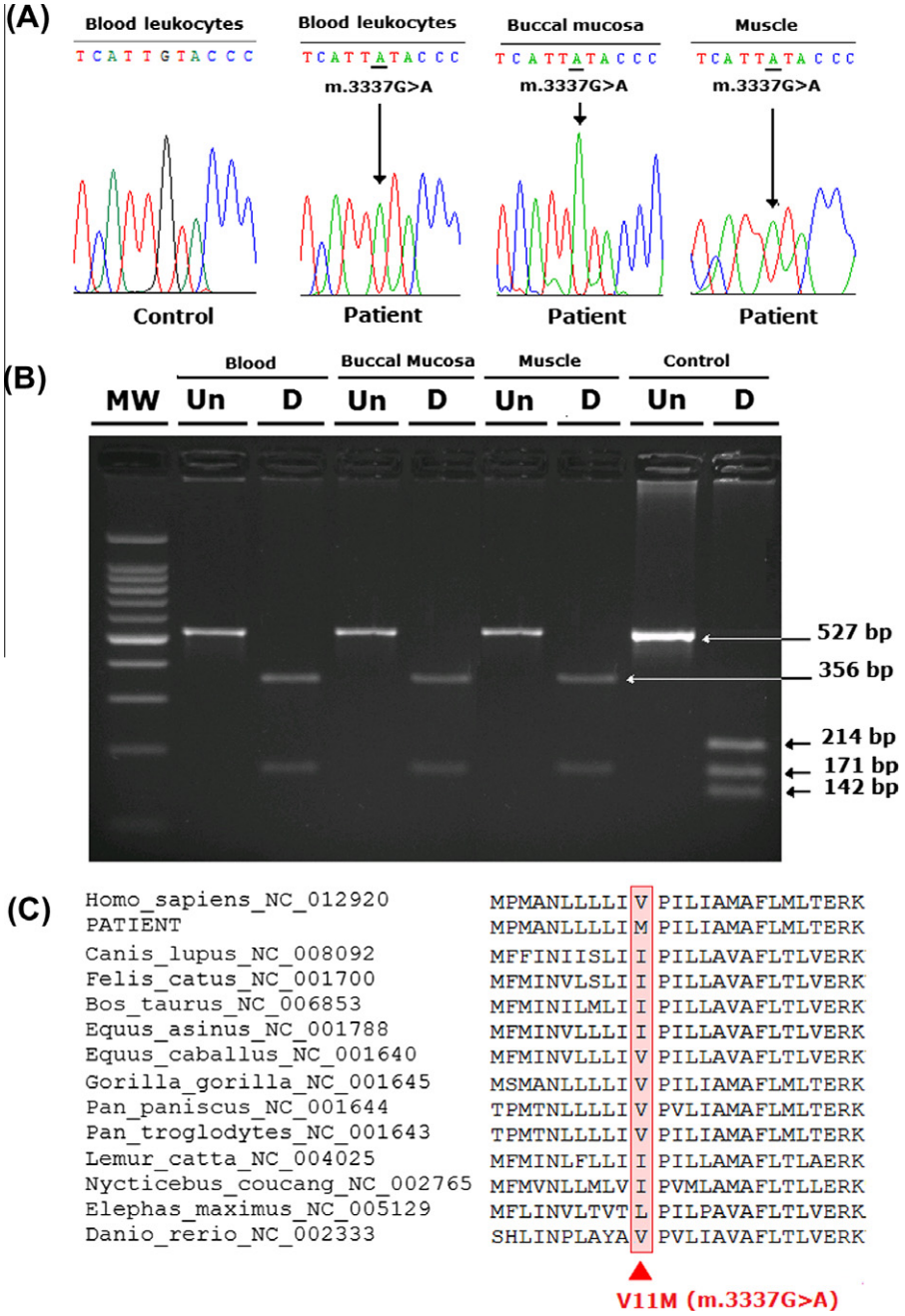

Fig. 1. (A) Sequence chromatograms showing the presence of the m.3337A>G mutation in the mitochondrial ND1 gene in the DNA extracted from blood leukocytes, buccal mucosa and skeletal muscle of the studied patient and its absence in a control. (B) PCR–RFLP analysis: a 527 bp PCR fragment is digested with *RsaI* restriction enzyme. The digestion in a mutated DNA shows 2 fragments of 356 and 171 bp, whereas the digestion of a wild-type DNA shows 3 fragments fragment of 214, 171 and 142 bp. MW: DNA Ladder 100 bp; Un: undigested PCR products; D: digestion with *RsaI* restriction enzyme in the extracted from blood leukocytes, buccal mucosa and skeletal muscle of the studied patient and also in a healthy control (C) alignment of the ND1 protein in different species showing the non-high conservation of the amino acid 11.