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Cytochrome C oxydase deficiency: *SURF1* gene investigation in patients with Leigh syndrome

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ABSTRACT

Leigh syndrome (LS) is a rare progressive neurodegenerative disorder occurring in infancy. The most common clinical signs reported in LS are growth retardation, optic atrophy, ataxia, psychomotor retardation, dystonia, hypotonia, seizures and respiratory disorders. The paper reported a manifestation of 3 Tunisian patients presented with LS syndrome. The aim of this study is the *MT[HYPHEN]ATP6* and *SURF1* gene screening in Tunisian patients affected with classical Leigh syndrome and the computational investigation of the effect of detected mutations on its structure and functions by clinical and bioinformatics analyses. After clinical investigations, three Tunisian patients were tested for mutations in both *MT-ATP6* and *SURF1* genes by direct sequencing followed by in silico analyses to predict the effects of sequence variation. The result of mutational analysis revealed the absence of mitochondrial mutations in *MT-ATP6* gene and the presence of a known homozygous splice site mutation c.516-517delAG in sibling patients added to the presence of a novel double het mutations in LS patient (c.752-18 A > C/c.751 + 16G > A). In silico analyses of theses intronic variations showed that it could alters splicing processes as well as *SURF1* protein translation. Leigh syndrome (LS) is a rare progressive neurodegenerative disorder occurring in infancy. The most common clinical signs reported in LS are growth retardation, optic atrophy, ataxia, psychomotor retardation, dystonia, hypotonia, seizures and respiratory disorders. The paper reported a manifestation of 3 Tunisian patients presented with LS syndrome. The aim of this study is *MT-ATP6* and *SURF1* genes screening in Tunisian patients affected with classical Leigh syndrome and the computational investigation of the effect of detected mutations on its structure and functions. After clinical investigations, three Tunisian patients were tested for mutations in both *MT-ATP6* and *SURF1* genes by direct sequencing followed by in silico analysis to predict the effects of sequence variation. The result of mutational analysis revealed the absence of mitochondrial mutations in *MT-ATP6* gene and the presence of a known homozygous splice site mutation c.516-517delAG in sibling patients added to the presence of a novel double het mutations in LS patient (c.752-18 A>C/ c.751+16G>A). In silico analysis of theses intronic vaiations showed that it could alters splicing processes as well as *SURF1* protein translation.

1. Introduction

Leigh syndrome (LS) (OMIM #256000), was firstly described in 1951 by Archibald Denis Leigh (1915–1998) Leigh syndrome, or subacute necrotizing encephalomyopathy, is a progressive neurological disease with an estimated prevalence of 1/40,000 births [1]. In children, this syndrome is the most common [2], which results in progressive developmental retardation with gradual, sometimes

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partially regressive regression, and it is defined by the bilateral and symmetrical presence of necrotic lesions of the basal ganglia, brain stem and cerebellum [3]. The disease typically begins in infants under one year of age, but there are rare cases of late onset until adulthood [4,5]. Early signs of the disease include dystonia, hypotonia with poor head strength, regression of motor acquisitions, ataxia, nystagmus, optic atrophy, respiratory disorders, and vomiting [6,7].

In 1965, a Leigh's syndrome patient presented a lactic acidosis with attacks of vomiting and failure to thrive, then a lack of pyruvate carboxylase activity in the liver of a patient with Leigh syndrome reflecting defective mitochondrial energy metabolism in 1968 [8]. In addition a cytochrome c oxidase deficiency was shown in another patient leading to an respiratory chain dysfunction, in 1977. Since that, it was indicated that Leigh syndrome could be a mitochondrial disorder. Therefore, this syndrome can be caused by defects of mitochondrial oxidative phosphorylation resulting from alterations of both mitochondrial and nuclear genes. Thus, Leigh syndrome may be inherited by autosomal recessive transmission, maternal transmission or may occur sporadically [9]. In fact mutations in over 60 genes have been reported in the mitochondrial DNA (mtDNA) as well as in the nuclear DNA. However, 75% of patients have a nuclear DNA mutation, and only 25% of cases are caused by mtDNA mutations [7].

SURF1 deficiency is the most frequent cause of the recessive inherited mitochondrial form of Leigh syndrome (LS) associated with cytochrome c oxidase. This complex is the fourth complex (complex IV) of the mitochondrial oxidative phosphorylation (OXPHOS) involved in electron transfer leading to finally the generation of the electrochemical gradient which used by complex V (ATP synthase) to synthesise ATP from ADP and inorganic phosphate. Several studies have discover A number of assembly factors and translation proteins are required for the biogenesis of the COX holoenzyme and it was demonstrated that SURF1 is a key player in the early assembly of COX [10,11].

More than 75% of patients are carriers of mutations in the *SURF1* gene, which encodes a complex IV assembly factor. Most often these mutations are responsible for the absence of a functional protein SURF1 and an alteration of the formation of the IV complex [12,13]. The *SURF1* gene is located on chromosome 9p34.2 in a group of “Surfeit” genes where the genomic structure of the region is well preserved. The major transcript has a size of 1 Kb and has 9 exons located in a range of 4 kb (isoform1). This gene encodes the SURF1 protein, which has 25% sequence homology with its yeast homologue SHY1. The SURF1 protein Comprises 300 amino acids and is located in the mitochondrial inner membrane [14]. The SURF1 protein contains three domains, one central and two transmembrane, N-terminal and C-terminal domains that play an important role in the function of the protein [15]. Although SURF1 is necessary for the assembly and maintenance of COX, its exact function is not yet well known.

2. Patients and methods

2.1. Patients

In this study, blood samples were collected from two sibling patients (P1, P2) belonging to a consanguine Tunisian family and a third isolated case (P3). The main clinical features of patients were summarized in (Table 1/Fig. 1).

2.1.1. Direct sequencing of the SURF1 gene

Genomic DNA was extracted from from peripheral blood leucocytes using standard procedures All coding exons, including flanking introns, in *SURF1* gene were amplified using specific primers previously described [16]. The PCR reaction was performed in GeneAmp PCR System 9700; (AppliedBiosystem). The reaction was carried out in a total volume of 25 μL, with 50 ng of genomic DNA, 8 pmol of each primer, 0.5 mM of each dNTP, 2 mM MgCl2, 1 × PCR buffer, and 1 U Taq DNA polymerase (Takara). PCR products were directly were then purified and sequenced with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (ABI PRISM/Biosystems).

2.1.2. Quantitative polymerase chain reaction

The mtDNA was quantitatively estimated on real-time amplification of ND4 fragments in the mtDNA genome, as described previously.10 To determine the overall abundance of mtDNA, the real-time amplification result of ND4 was compared with that of

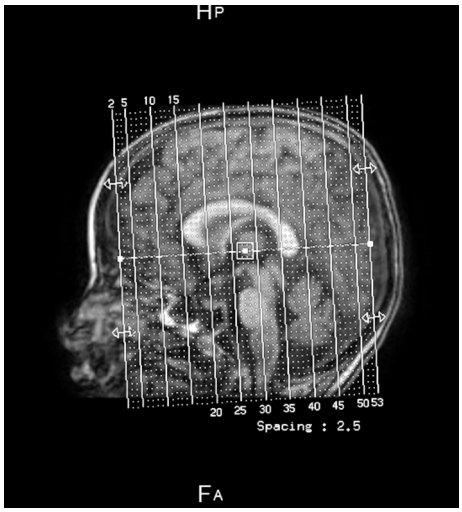

Fig. 1. Magnetic resonance images of the brain representing typical Leigh pathology: MRI showed symmetric hyperintense signal involving the head of the caudate nucleus, and the putamen.

Table 1
Clinical features of studied patients.

Patient	Sex	Age (years)	Lactate (blood) nmol/l	Family history	Clinical signs
P1	M	7	3.9(blood)	Negative	They were born from consanguine parents First signs were observed at the age of 1 year in P1 and at age of 9 months in P2
P2	F	3	3.1(blood)	Negative	including psychomotor and neurodevelopment delay; optic atrophy; severe difficulties to walk; hypotonia;bilateral hearing loss, sensorimotor polyneuropathy MRI showed symmetric hyperintense signal involving the head of the caudate nucleus, the putamen. They are still alive at the age of 10 and 3 years, respectively.
P3	M	2	5.2(blood) 7.4(LCR)	Unknown	He was born from non consanguine parents. First signs were observed a age of 1 year: global hypotonia, failure to thrive, psychomotor and neurodevelopment delay and an elevated lactate pyruvate ratio (0.22;blood,0.26;LCR) MRI showed bilateral abnormal signal intensity in the basal ganglia and cerebellar atrophy

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