Short communication

Identification of a novel deletion in SURF1 gene: Heterogeneity in Leigh syndrome with COX deficiency

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ABSTRACT

Leigh syndrome (LS) is a rare, progressive neurodegenerative mitochondrial disorder of infancy. It is a genetically heterogeneous disease. The mutations in SURF1 gene are the most frequently known cause. Here two cases of LS likely caused by SURF1 gene variants are reported: a 39-year-old male patient with a novel homozygous deletion (c.-11_13del), and a case of a 6-year-old boy with the same deletion and a nonsense mutation (c.868dupT), both in heterozygosity. Blue native PAGE showed absence of assembled complex IV. This is the first report of a variant that may abolish the SURF1 gene initiation codon in two LS patients.

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1. Introduction

Leigh syndrome (LS) (OMIM #256000), first described in 1951, is characterized by focal, symmetrical, and necrotic lesions in the thalamus, the brain stem, and the posterior columns of the spinal cord (Munnich et al., 1996; Munnich et al., 2012). The neuropathological diagnosis has been replaced by brain magnetic resonance imaging (MRI), which exhibits typical lesions (Tiranti et al., 1999). Usually, LS presents at an early age with psychomotor regression, pyramidal and extrapyramidal symptoms and signs of brain stem dysfunction, such as apnea and other respiratory rhythm abnormalities. Several inborn errors of energy metabolism, such as pyruvate dehydrogenase and mitochondrial respiratory chain (MRC) deficiencies have been associated with LS, although in some cases the cause cannot be identified. One of the most common enzymatic deficiencies associated to LS is cytochrome c oxidase (COX, complex IV) dysfunction (Piekutowska-Abramczuk et al., 2009).

Mutations in nuclear genes – SURF1, SCO1, SCO2, COX10, COX15, LRPPRC, PDHA1 – have been associated to LS (Finsterer, 2008). According to Agostino et al. (2003), the most common genetic cause in LS patients with complex IV deficiency involves SURF1 gene. The association between SURF1 mutations and LS seems rather specific, since no alterations in SURF1 are usually detected in complex IV deficiency patients with other clinical presentations (Agostino et al., 2003). The SURF1 gene is located at chromosome 9p34, in a cluster referred as the "surfeit" genes that are highly conserved throughout evolution (Duhig et al., 1998). It encodes a mitochondrial inner membrane protein with 300 amino acids (Piekutowska-Abramczuk et al., 2009) that is involved in the assembly and maintenance of complex IV (Kovárová et al., 2012).

2. Materials and methods

2.1. Clinical subjects

This study was conducted during the diagnostic investigation of the genetic cause of the disease and informed consent was obtained from the participants or their legal representatives, as recommended by the local Ethics Committee, following the Tenets of the Helsinki Declaration.

2.2. SURF1 gene sequencing

After extracting genomic DNA from whole blood of the affected patients and their parents, SURF1 sequencing was performed following standard techniques. The identified deletion (according to NM_003172) was confirmed by PCR-RFLP (Supplementary File). The newly described deletion has been submitted to ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/).
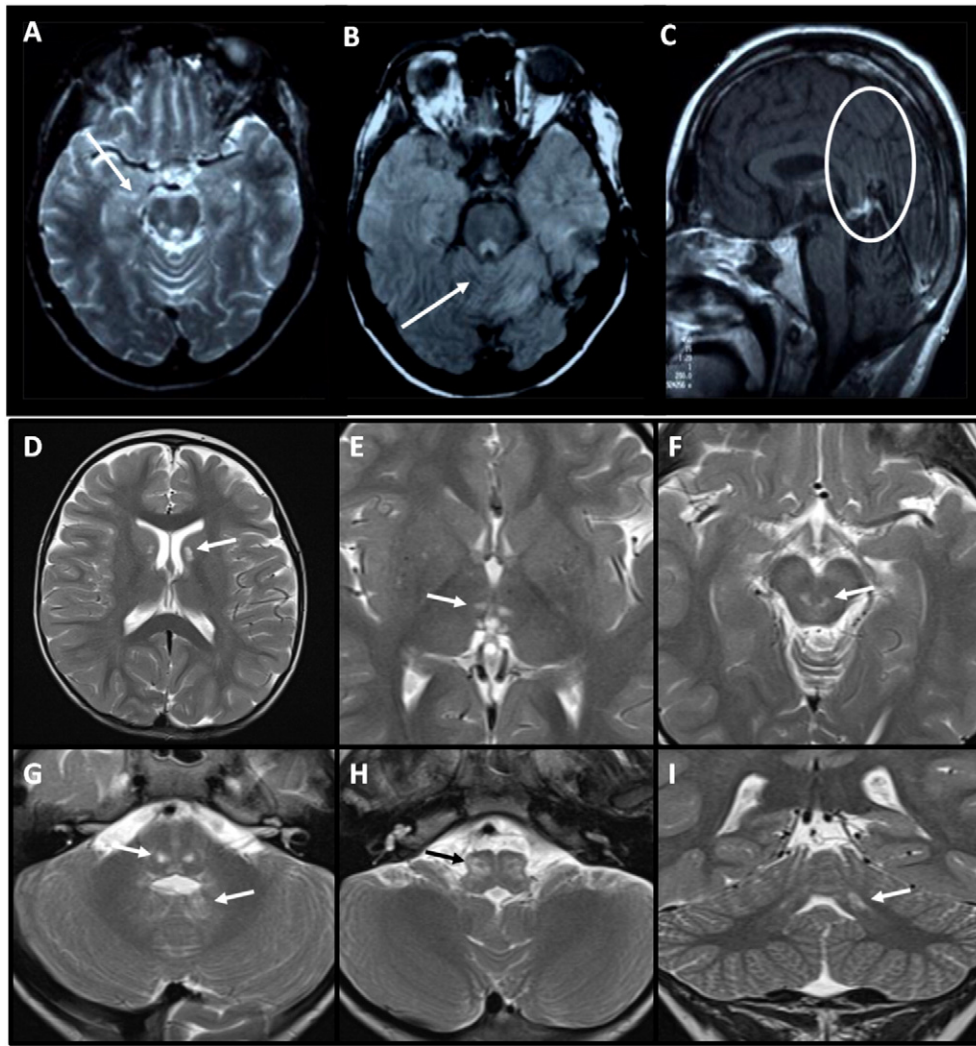
Fig. 1. Radiological features of patient 1: axial T2-weighted images (T2WI) (A–C) showing (A) hyperintense lesion (arrow) in the head of the left caudate nucleus; (B) symmetrical involvement of the midbrain tegmentum (arrow); (C) involvement of periaqueductal gray matter (circle). Radiological features of patient 2: Axial T2WI (D–I) showing bilateral hyperintense lesions (indicated by arrows) of the head of the caudate nuclei (D) and the thalamic posteromedial ventral nuclei (E). Symmetrical involvement of the dorsal midbrain (F), dorsal pons and deep cerebellar white matter (G) and olivary nuclei (H) is also noted. Coronal T2WI shows additional lesion of the left dentate nucleus.

2.3. Blue native PAGE (BN–PAGE) analysis

Fibroblasts from the patients with *SURF1* mutations were used to evaluate assembly of MRC complex IV monomers, using the methodology previously reported for BN-PAGE analysis (Calvaruso et al., 2008) with modifications (Supplementary File).
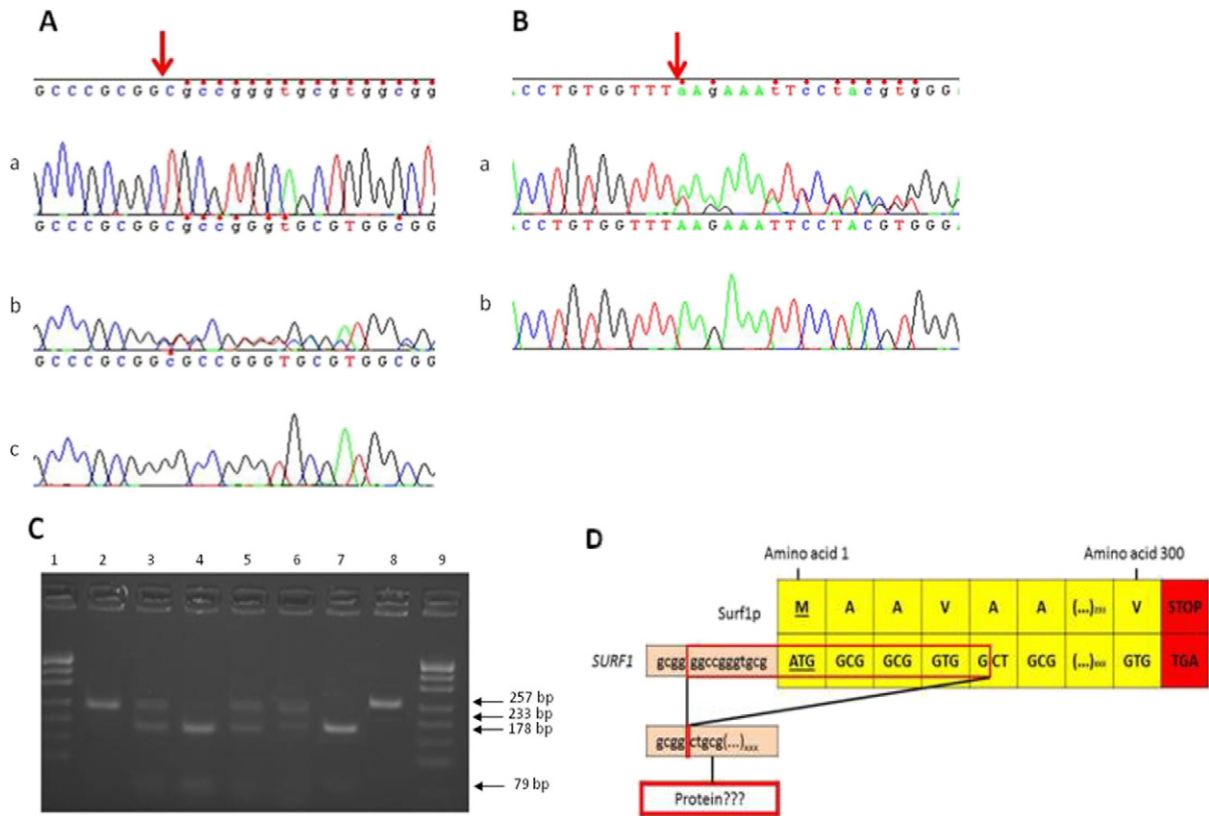
3. Results

Patient 1 is a Caucasian male patient, born at term in 1976, from a healthy non-consanguineous couple. Family history is irrelevant, except for diabetes mellitus on maternal lineage. At 2 years of age unilateral strabismus was noticed and by 3–4 years dysarthria, ataxia, kinetic tremor and slight hypotonia developed. At 8 years of age, an acute deterioration with shock, coma, central apnea and ophthalmoparesis emerged, after proctated vomiting. Etiological investigation, namely infectious and metabolic, was inconclusive. Computed tomography of the brain was normal and MRI showed moderate cerebellum atrophy. Progressive recovery ensued, except for right hemidystonia. At 21 years of age the patient experienced a sudden, non painful, loss of vision and the diagnosis of retinal central vein thrombosis was made. Thrombophilia screening was negative. Laboratory investigation revealed increased lactate and lactate/pyruvate ratio, moderate complex

IV deficiency in lymphocytes – 29.7% of average control value corrected for citrate synthase (criteria according to Grazina, 2012). Five years later, in the sequence of an acute pharyngitis, this patient developed central hipoventilation lead to respiratory insufficiency with need for chronic mechanical ventilation. Brain-MRI revealed the typical lesions observed in LS (Fig. 1A–C). Muscle biopsy study disclosed no histology abnormalities and low complex IV activity – 22.5% of average control value corrected for citrate synthase (criteria according to Grazina, 2012).

The *SURF1* gene investigation revealed a homozygous deletion of 24 nucleotides, c.-11_13del (Fig. 2A and C). This alteration was absent in 200 DNA samples of healthy subjects and it was not reported in any public SNP databases or in recent publications (Lee et al., 2012; Wedatilake et al., 2013). The deletion is located in a highly conserved region (Fig. 2E) and causes the loss of ATG codon, essential for the protein synthesis initiation (Fig. 2D). Patient's 1 mother *SURF1* screening identified the deletion in heterozygosity; the DNA sample from the father was not available for analysis.

Patient 2 is a 6-year-old boy, born in 2009, at term from healthy, non-consanguineous parents, with irrelevant family history. At 3 years of age he presented in coma with respiratory acidosis, in the sequence of rhinopharyngitis and vomiting. He had been followed in the outpatient clinic from two years of age due to failure to thrive, strabismus,
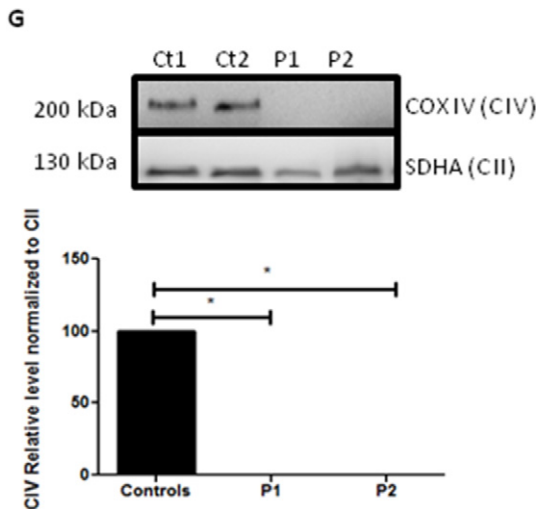
E

Homo sapiens	aagcgcccgcggggccgggtgcgATGGCGGCGGTGGCTGCGTTGCAGC
Pan troglodytes	aagcgcccgcggggctgggtgcgATGGCGGCGGGGGCTGCGTCGCAGC
Gorilla gorilla	aagcgctcgcggggctgggtgcgATGGCGGCGGGGGCTGCGTCGCAGC
Pongo abelii	aagcgcccgcggggctgggtgcgATGGCGGCGGGGGCTGCGTCGCCCC
Macaca mulatta	aagagcccgcggggccgggtgcgATGGCGGCGGTGGCTGCGCTGCAGC
Patients 1 and 2	aagcgcccgcgg----------------------CTGCGTTGCAGC

F

Homo sapiens	TACCTGTGGTTT-AAGAAATTCCTA	YLWFKKFL
Pan troglodytes	TACCTGTGGTTT-AAGAAATTCCTA	YLWFKKFL
Gorilla gorilla	TACCTGTGGTGT-AAGAAATTCCTA	YLWFKKFL
Pongo abelii	TACCTCTGGTTT-AAGAAATTCCTA	YLWFKKFL
Macaca mulatta	TACCTGTGGTTT-AAGAAATTCCTA	YLWFKKFL
Patient 2	TACCTGTGGTTT**T**AAGAAATTCCTA	YLWF**X**

and ataxia, with loss of motor milestones. The brain-MRI revealed lesions typical of LS and Magnetic Resonance Spectroscopy, lactate peaks (Fig. 1D–I).

Laboratory investigation disclosed normal lactate, glycaemia, creatinine kinase, amino acids and organic acids. Later on, increased lactate and lactate/pyruvate ratio were detected. Complex IV activity in lymphocytes was 20.2% of average control value corrected for citrate synthase (criteria according to Grazina, 2012). Isolated complex IV deficiency was detected in the muscle biopsy – 18.4% of average control value corrected for citrate synthase (criteria according to Grazina, 2012). Investigation of SURF1 revealed a heterozygous deletion, c.-11_13del (Fig. 2A), and a heterozygous T duplication, c.868dupT (Fig. 2B). The c.868dupT is absent in 200 samples of healthy subjects and is located in a highly conserved region (Fig. 2F). This alteration was previously reported in LS patients (Tiranti et al., 1998; Kinghorn et al., 2013; Aulbert et al., 2014). Each parent is a carrier (heterozygous) for one of those alterations (c.-11_13del in the father and c.868dupT in the mother).

The BN–PAGE experiments demonstrated the absence of assembled complex IV in patients' samples (Fig. 2G).

4. Discussion

The SURF1 is embedded in a cluster of 6 housekeeping genes (the surfeit locus, SURF1 to 6) and the structure of this cluster is unique in the mammalian genome (Huxley and Fried, 1990). This basic structure has been conserved during evolution, suggesting that it has a functional importance in coordinating gene expression regulation. So far, >78 mutations were found in SURF1, and about 80% probably lead to the production of truncated proteins, mainly due to aberrant splicing, frameshift, deletions or nonsense mutations (Wedatilake et al., 2013). The majority of SURF1 mutations previously associated to LS, are located in exon 8, suggesting that this region has an important impact in the protein function or it is a hot spot for the occurrence of mutations (Lee et al., 2012).

So far, the deletion found is a novel variant and it is one of the largest rearrangements reported for SURF1 gene. The precise functional consequences of the c.-11_13del are difficult to predict, but with the loss of the first codon, it is possible that another ATG sequence may be recognized as the protein initial codon, probably significantly altering the SURF1 protein (Surf1p). Nevertheless, the BN-PAGE results demonstrating the absence of assembled complex IV in patients' fibroblasts' samples, reinforce and support the deleterious role of the mutations, particularly the novel deletion c.-11_13del, herein described.

In 1999, Tiranti et al. evaluated the expression of SURF1 gene at the mRNA level in LS patients carrying different mutations of the gene. They have concluded that different mutations spanning the first 770 nucleotides of SURF1 lead to the absence of transcript. These results suggested that alterations in the initial region of the gene are probably associated with severe mRNA instability (Tiranti et al., 1999), which might be also occurring with the identified deletion SURF1 transcripts of the present work.

It is important to emphasize the current age of patient 1: 39 year-old. To our knowledge, it is the oldest patient ever reported with LS associated to SURF1 mutations. Patients with SURF1-associated LS have a poor prognosis and usually die in the first decade of life (Lee et al., 2012). According to Aulbert et al. in 2014, a total of 15 patients with survival higher than 14 years have been reported and the oldest patient

with LS associated to SURF1 mutations was a woman with 22 years old (Lee et al., 2012; Aulbert et al., 2014). This patient had two mutations that were predicted to generate truncated proteins at amino acid positions 248 and 279. According to the authors, the milder phenotype could be explained by the fact that those alterations occur near the C-terminus of the protein and it could have some residual function (Lee et al., 2012).

Considering that patient 1 has a predictably deleterious SURF1 deletion in homozygosity, his long survival can be due to a possible compensatory mechanism that counterbalances Surf1p function in the assembly of complex IV (Shoubridge, 2001; Dell'Agnello et al., 2007). Kovárová et al. detected two dominant complex IV forms in SURF1-mutant patients' fibroblasts: a large amount of COX1 assembly intermediates and the majority of fully assembled complex IV were in I-III$_2$-IV$_n$ supercomplexes. By contrast, the human control fibroblasts complex IV is mainly found as a monomer (Kovárová et al., 2016). This report supports the lack of assembled complex IV (as a monomer) found in P1 and P2 patients and may explain the residual activity of complex IV (% of control activity, respectively for P1 and P2: 22.5% and 18.4% - muscle; 26.4% and 25.8% - fibroblasts).

Patient 2 has a compound heterozygous mutation with the c.-11_13del and the c.868dupT in exon 9. This duplication is predicted to generate a stop codon in amino acid 291 (p.Lys291X), resulting in a protein possibly smaller in 9 amino acids compared to the normal Surf1p. According to Tiranti et al. (1999), this alteration generates a truncated protein with residual complex IV activity (Tiranti et al., 1999). The reported deficiency may be due to a loss of Surf1p conserved C-terminus transmembrane domain integrity. The phenotype of patient 2 is expected to be due to the combination of the compound heterozygous mutations found.

In conclusion, in this work two alterations are reported: the c.868dupT and a novel deletion (c.-11_13del) in the 5′UTR region and the initially coding nucleotides of SURF1 gene. This deletion is the first alteration reported in this region and possibly leading to the loss of the ATG codon, although the consequences for Surf1p expression will only be clear after performing additional functional studies. Patient 1 presents only the novel deletion in homozygosity and, to our knowledge, this is the oldest patient reported with SURF1 mutations possibly associated to LS. The long survival of this patient can be possibly explained by an alternative compensatory mechanism that counterbalances Surf1p function in the complex IV assembly.

Conflicts of interest

The authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.mito.2016.10.004.

Fig. 2. (A) Electropherogram of the region surrounding the deletion c.-11_13del in patient 1 (a), patient 2 (b) and a control (c); (B) electropherogram of the region surrounding c.868dupT alteration in patient 2 (a) and a control (b); (C) PCR-RFLP of c.-11_13del mutation: 1 - pUC19 DNA/MspI (HpaII) Marker, 23 (Fermentas, Vilnius, Lithuania); 2 - Patient 1; 3 - Patient's 1 mother; 4 - Patient's 2 mother; 5 - Patient 2; 6 - Patient's 2 father; 7 - Control sample, 8 - Non digested; 9 - pUC19 DNA/MspI (HpaII) Marker, 23 (Fermentas, Vilnius, Lithuania); (D) Schematic representation of Surf1p, SURF1 gene 5′UTR and initial codons with the deletion c.-11_13del marked with a box, and SURF1 gene representation of the remaining nucleotide sequence; (E) Nucleotide evolutionary conservation study for SURF1 gene c.-11_13del mutation in primate species. Lowercase indicates the 5′UTR region, − indicate deleted nucleotides and uppercase indicates the exon 1; (F) Nucleotide and amino acid evolutionary conservation study for SURF1 gene c.868dupT surrounding region in exon 9 and Surf1p p.Lys291X. Nucleotide underlined and in bold indicate the duplicated nucleotide, "X" indicates the stop codon in amino acid sequence; (G) Study of complex IV assembly by BN-PAGE of fibroblasts' samples (from controls, Ct, and patients, P1 and P2); *p < 0.05.

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