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Original article

Exome sequencing identifies complex I NDUFV2 mutations as a novel cause of Leigh syndrome

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ABSTRACT

Background: Two siblings with hypertrophic cardiomyopathy and brain atrophy were diagnosed with Complex I deficiency based on low enzyme activity in muscle and high lactate/pyruvate ratio in fibroblasts.
Methods: Whole exome sequencing results of fibroblast gDNA from one sibling was narrowed down to 190 SNPs or In/Dels in 185 candidate genes by selecting non-synonymous coding sequence base pair changes that were not present in the SNP database.
Results: Two compound heterozygous mutations were identified in both siblings in *NDUFV2*, encoding the 24 kDa subunit of Complex I. The intronic mutation (c.IVS2 + 1delGTAA) is disease causing and has been reported before. The other mutation is novel (c.669_670insG, p.Ser224Valfs*3) and predicted to cause a pathogenic frameshift in the protein. Subsequent investigation of 10 probands with complex I deficiency from different families revealed homozygosity for the intronic c.IVS2 + 1delGTAA mutation in a second, consanguineous family. In this family three of five siblings were affected. Interestingly, they presented with Leigh syndrome but no cardiac involvement. The same genotype had been reported previously in a two families but presenting with hypertrophic cardiomyopathy, trunk hypotonia and encephalopathy.

Abbreviations: bp, base pair; In/Del, insertion/deletion; LHON, Leber's Hereditary Optic Neuropathy; MELAS, mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes; MERFF, myoclonic epilepsy associated with ragged red fibers; NARP, neuropathy, ataxia, and retinitis pigmentosa; mtDNA, mitochondrial DNA; SNP, single nucleotide polymorphism; RRF, ragged red fibers.

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Conclusion: We have identified *NDUFV2* mutations in two families with Complex I deficiency, including a novel mutation. The diagnosis of Leigh syndrome expands the clinical phenotypes associated with the c.IVS2 + 1delGTAA mutation in this gene.

1. Introduction

Complex I (NADH: ubiquinone oxidoreductase) is the first enzymatic component of the mitochondrial respiratory chain, and the largest enzyme complex. It comprises 45 subunits, of which 38 are encoded by the nuclear genome and seven encoded by the mitochondrial genome. Molecular nuclear mutations causing complex I deficiency have been identified in 19 of the subunits.[1–53] As many as 17 proteins required for assembly of the complex have been identified, with causative molecular mutations identified in ten of these.[54–85] Complex I deficiency can present as a number of clinical phenotypes: fatal infantile lactic acidosis, Leigh syndrome or other forms of encephalopathy, neonatal cardiomyopathy and leukodystrophy are the most prevalent, often in combination with cardiomyopathy.[86]

We have identified mutations in the *NDUFV2* subunit of complex I in two families. The identification of a novel mutation expands the genotypic information associated with complex I deficiency, and the identification of two siblings with a homozygous mutation that has been previously published, allows us to conclude the phenotypic presentation associated with this mutation is broader than initially suggested.[52,53]

2. Materials and methods

2.1. Patients

All procedures were carried out with informed parental consent and with approval from The Hospital for Sick Children's research ethics board.

2.1.1. Family I (Fig. 1a)

The parents of this family are non-consanguineous, and of Caucasian origin. There is no family history of note. I/1: A male, unaffected sibling.

I/2 Proband: A male, born at 36 weeks, and weighing 2.46 kg. He was admitted on day 7 (weight 2.6 kg) for jaundice and feeding issues. At 4 weeks he was re-admitted with jaundice and dehydration but no cardiac disease was noted. At 11 weeks he suffered a sudden collapse followed by cardiac arrest. His lactate was 28.5 mM but returned to normal (2.5 mM) in 24 h with resuscitation. Echocardiography revealed left-ventricular hypertrophic cardiomyopathy with a left-ventricular outlet flow gradient of 30 mmHg. A 'mito cocktail' of coenzyme Q, riboflavin, niacin, succinate, and carnitine plus inotropes was administered. At 15 months, before he passed away, muscle histopathology showed mild variation in fiber size, scattered atrophic fibers, and subsarcolemmal prominence of mitochondria on modified Gomori-Trichrome stain, but no ragged red fibers (RRF) or glycogen accumulation. Electron microscopy revealed increased mitochondrial numbers with whorled cristae. A CT scan showed mild brain atrophy, but no basal ganglia or thalamic changes to suggest Leigh disease. MRI was not available. MtDNA analysis from blood leukocytes for MELAS (m.3243 and m.3271); MERFF (m.8344) and NARP (m.8993) were

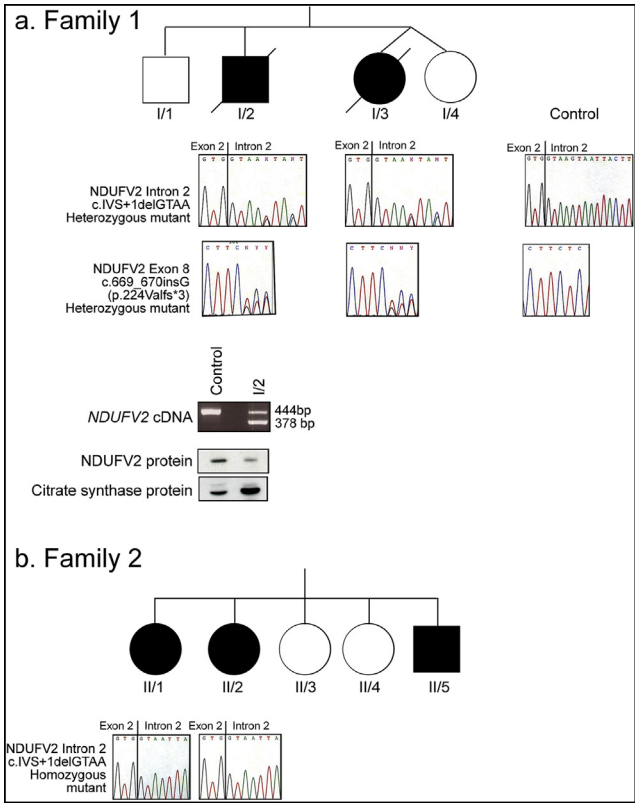

Fig. 1 – (a) The pedigree for Family 1 is shown. Sequence chromatograms are depicted for the *NDUFV2* gDNA region flanking the intron 2 and exon 8 compound heterozygous mutations (patients I/2 and I/3) and control. Amplification of full length *NDUFV2* cDNA is shown for I/2, as well as immunoblotting of 20 μg fibroblast mitochondria with antibodies raised against NDUFV2 and citrate synthase proteins. (b) The pedigree for Family 2 is shown. Sequence chromatograms are depicted for the *NDUFV2* gDNA region flanking the intron 2 homozygous mutations in (patients II/1 and II/2).