Leigh Syndrome Associated With Mitochondrial Complex I Deficiency Due to a Novel Mutation in the NDUFS1 Gene

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Background: Mutations in the nuclear-encoded subunits of complex I of the mitochondrial respiratory chain are a recognized cause of Leigh syndrome (LS). Recently, 6 mutations in the NDUFS1 gene were identified in 3 families.

Objective: To describe a Spanish family with LS, complex I deficiency in muscle, and a novel mutation in the NDUFS1 gene.

Design: Using molecular genetic approaches, we identified the underlying molecular defect in a patient with LS with a complex I defect.

Patient: The proband was a child who displayed the clinical features of LS.

Results: Muscle biochemistry results showed a complex I defect of the mitochondrial respiratory chain. Sequencing analysis of the mitochondrial DNA-encoded ND genes, the nuclear DNA-encoded NDUFS1, NDUFS4, NDUFS6, NDUFS7, NDUFS8, and NDUFAB1 genes, and the complex I assembly factor CIA30 gene revealed a novel homozygous L231V mutation (c.691C→G) in the NDUFS1 gene. The parents were heterozygous carriers of the L231V mutation.

Conclusions: Identifying nuclear mutations as a cause of respiratory chain disorders will enhance the possibility of prenatal diagnosis and help us understand how molecular defects can lead to complex I deficiency.

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Leigh Syndrome (LS) (Online Mendelian Inheritance in Man 256000) is a devastating neurodegenerative disorder characterized neuropathologically by focal bilaterally symmetrical lesions, especially in the thalamus and brainstem regions, and clinically by psychomotor retardation, respiratory difficulties, nystagmus, ophthalmoplegia, optic atrophy, ataxia, and dystonia.1 In most patients, mitochondrial respiratory chain defects and pyruvate dehydrogenase complex deficiency are the underlying causes of the disease.2 Mitochondrial respiratory chain complex I (nicotinamide adenine dinucleotide:ubiquinone oxidoreductase) contains at least 46 subunits, 7 of which are encoded by mitochondrial DNA (mtDNA).3 Various mutations in a few subunits of complex I encoded by nuclear DNA (nDNA) (NDUFV1, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS5, and NDUFS6)3,4 are associated with LS or Leigh-like disease in patients with complex I deficiency. The NDUFS1 gene encodes the largest (75-kDa subunit) protein of complex I.11 Recently, 6 mutations in the NDUFS1 gene were identified in 3 families with LS or Leigh-like disease and complex I deficiency (Online Mendelian Inheritance in Man 157655).5 Herein, we describe a Spanish patient with LS, complex I deficiency in muscle, and a novel mutation in the NDUFS1 gene.

Methods

Report of a Case

The first child (a girl) of healthy nonconsanguineous parents (aged 30 years) of Spanish origin was born after a term pregnancy (birth weight, 3560 g). She was hospitalized at age 8½ months for recurrent episodes of vomiting, floppiness, and growth retardation. She presented with irritability, horizontal nystagmus, and generalized hypotonia. Tendon reflexes were hyperactive and symmetric. Babinski sign was negative. An electroencephalogram displayed a normal pattern. Brain magnetic resonance imaging showed bilateral lesions affecting the substantia nigra and midbrain. Cardiologic and ophthalmologic examination findings were normal. Laboratory data revealed increased lactate levels in blood (24 mg/dL; normal, <20 mg/dL) and in cerebrospinal fluid.

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The activities of respiratory chain complexes in muscle showed a single defect of nicotinamide adenine dinucleotide:ubiquinone oxidoreductase (complex I), accounting for 25% of the mean of the control subjects (Table). Given the clinical picture and the biochemical findings, we searched for the underlying molecular alteration of this defect. Sequencing analysis of the genes listed in the “Methods” section showed a novel homozygous missense mutation (L231V) that replaces a leucine by a valine in the 231 amino acid residue of the protein as a result of a c.691C→T transition in exon 8 of the NDUFS1 gene (Figure). Additional nucleotide changes were not found. The parents were heterozygous carriers of the L231V mutation.

**RESULTS**

Mutations in the nDNA-encoded complex I NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS5, and NDUFS8 genes have been documented in patients with LS, although often the underlying molecular defect remains unknown. In 3 nonrelated families, Bénit et al. identified 6 mutations in the NDUFS1 gene, which encodes the largest subunit of complex I. Interestingly, 3 of these mutations (amino acids 222, 241, and 252) lie in a highly evolutionarily conserved stretch of the protein encompassing the most C-terminal cysteine residue, potentially involved in the ligation of iron-sulfur clusters.

We describe a Spanish girl with LS, whose younger brother died of a similar condition. The proband had a complex I defect in muscle and harbored a novel homozygous missense mutation (L231V) in the NDUFS1 gene. The mutation was consistently heterozygous in blood DNA from the healthy parents, suggesting autosomal recessive inheritance. Several lines of evidence support the pathogenicity of the mutation, including the following: (1) the patient had a single complex I defect in muscle; (2) it was the only nucleotide change found in the entire coding region and in the intron and exon boundaries of the gene; (3) no additional pathogenic mutations were found in the other complex I genes analyzed; (4) the mutation was absent in 200 alleles from 100

**COMMENT**

An appropriate institutional review board approved this work, and informed consent was obtained from the child’s parents. Respiratory chain enzymes in muscle homogenate were measured by methods reported elsewhere. DNA was isolated from muscle and blood from the patient and from blood from her parents. The mtDNA-encoded ND subunits were amplified using suitable primers. The coding region and exon and intron boundaries of the nuclear-encoded complex I subunits of the NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS5, NDUFS8, and NDUFA1 genes, as well as the complex I assembly factor CIA30 gene, were amplified as previously described or by using novel intronic primers. Polymerase chain reaction products were purified by electrophoresis in 2% agarose gel and sequenced directly, using the ABI PRISM dRhodamine Terminator Cycle Sequencing Kit in an ABI PRISM 310 genetic analyzer (Applied Biosystems, Foster City, Calif). Nucleotide changes were further confirmed by polymerase chain reaction–restriction fragment length polymorphism methods (Figure). One hundred healthy control subjects (200 alleles) were screened by polymerase chain reaction–restriction fragment length polymorphism methods to rule out the presence of the mutation in the healthy population. Unfortunately, tissue specimens were not available from the proband’s brother.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Patient</th>
<th>Control Subjects, Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(n = 100)</td>
</tr>
<tr>
<td>Nicotinamide adenine dinucleotide:ubiquinone oxidoreductase</td>
<td>5.0</td>
<td>20.0 (4.5)</td>
</tr>
<tr>
<td>Succinate dehydrogenase</td>
<td>8.7</td>
<td>10.3 (3.5)</td>
</tr>
<tr>
<td>Decylubiquinol-cytochrome c oxidoreductase</td>
<td>50.7</td>
<td>63.0 (18.0)</td>
</tr>
<tr>
<td>Cytochrome c oxidase</td>
<td>22.8</td>
<td>42.0 (12.1)</td>
</tr>
</tbody>
</table>

*Data are given as percentage of citrate synthase activity. Activities were measured as nanomoles per minute per milligram of protein.

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We describe a family with LS, complex I deficiency, and a novel mutation in the NDUF51 gene. The rapidly progressive nature of the disease, absence of effective treatment, and commonly fatal course of the disease make prenatal diagnosis a valuable tool in families with this condition. Identifying nuclear mutations as a cause of mitochondrial respiratory chain disorders will enhance the possibility of prenatal diagnosis and help us understand how molecular defects can lead to complex I deficiency.

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