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

Miguel A. Martín, PhD; Alberto Blázquez, BSc; Luis G. Gutierrez-Solana, MD; Daniel Fernández-Moreira, PharmB; Paz Briones, PhD; Antoni L. Andreu, MD, PhD; Rafael Garesse, PhD; Yolanda Campos, PhD; Joaquín Arenas, PhD

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 NDUFV1, NDUFS1, NDUFS2, 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.

Arch Neurol. 2005;62:659-661

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, ophthalmoparesis, 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).2 Various mutations in a few subunits of complex I encoded by nuclear DNA (nDNA) (NDUFV1, NDUFV2, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS7, and NDUFS8)3-10 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.
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 NDUF31 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 healthy control subjects (200 alleles).

**RESULTS**

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→G transition in exon 8 of the NDUF31 gene (Figure). Additional nucleotide changes were not found. The parents were heterozygous carriers of the L231V mutation.

**COMMENT**

Mutations in the nDNA-encoded complex I NDUFV1, NDUFV2, NDUF31, NDUF52, NDUF53, NDUF54, NDUF57, and NDUF58 genes have been documented in patients with LS, although often the underlying molecular defect remains unknown. In nonrelated families, Bénit et al. identified 6 mutations in the NDUF31 gene, which encodes the largest subunit of complex I. Interestingly, 3 of these mutations (amino acids 222, 241, and 252) lie in a highly evolutionary 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 NDUF31 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 healthy control subjects (200 alleles).

**Table. Activities of Mitochondrial Respiratory Chain Complexes in Muscle Homogenate**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Control Subjects, Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotinamide adenine dinucleotide:ubiquinone oxidoreductase</td>
<td>5.0 (4.5)</td>
</tr>
<tr>
<td>Succinate dehydrogenase</td>
<td>8.7 (3.5)</td>
</tr>
<tr>
<td>Decylubiquinol-cytochrome c oxidoreductase</td>
<td>50.7 (18.0)</td>
</tr>
<tr>
<td>Cytochrome c oxidase</td>
<td>22.8 (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.*

**BIOCHEMICAL AND MOLECULAR GENETIC STUDIES**

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 NDUFV1, NDUF31, NDUF52, NDUF53, NDUF54, NDUF57, NDUF58, and NDUFAB1 genes, as well as the complex I assembly factor CL30 gene, were amplified as previously described or by using novel intronic primers. Polymere 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.
healthy controls of similar ethnic background; and (5) although the amino acid substitution does not result in a polarity change, the mutation is highly conserved during evolution and is situated in a region of the protein subunit where 3 other mutations were found (Figure).3

Reliable prenatal diagnosis is a difficult task in many cases of mitochondrial respiratory chain disorders. The identification of mutations in nuclear genes in families with a clear-cut pattern of autosomal recessive inheritance makes it possible to predict diagnosis in the fetus.16,17

In addition to this family, we analyzed the nuclear-encoded mitochondrial genes described herein in a series of 13 pediatric patients with LS or Leigh-like disease with a complex I defect and found no additional patients with mutations in these genes. In other reports, frequencies of patients with these mutations ranged between 17% and 25%.2,5 Only 7.7% of our patients with LS or Leigh-like disease and an isolated complex I defect harbor mutations in these genes.

CONCLUSIONS

We describe a family with LS, complex I deficiency, and a novel mutation in the NDUFAS1 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.

Accepted for Publication: April 8, 2004.

Author Affiliations: Centro de Investigación, Hospital Universitario 12 de Octubre (Drs Martín, Campos, and Arenas and Messrs Blázquez and Fernández-Moreira), Servicio de Neurología, Hospital Infantil Universitario Niño Jesús (Dr Guiterrez-Solana), Instituto Investigaciones Biomédicas “Alberto Sols” Universidad Autónoma de Madrid—Consejo Superior de Investigaciones Científicas, Departamento de Bioquímica, Facultad de Medicina, Universidad Autónoma de Madrid (Dr Garesse), Madrid; and Institut de Bioquímica Clínica, Corporació Sanitaria Clinic y Consejo Superior de Investigaciones Científicas (Dr Briones) and Centre d’Investigacions en Bioquímica i Biologia Molecular, Hospital Universitari Vall d’Hebron (Dr Andreu), Barcelona, Spain. Dr Martin and Mr Blázquez contributed equally to this work.

Correspondence: Joaquin Arenas, PhD, Centro de Investigación, Hospital Universitario 12 de Octubre, Avenida de Córdoba sin número, 28041 Madrid, Spain (jarenas.hdoc@salud.madrid.org).

Author Contributions: Study concept and design: Martín, Blázquez, and Arenas. Acquisition of data: Martín, Blázquez, Gutiérrez-Solana, Fernández-Moreira, Briones, and Campos. Analysis and interpretation of data: Martín, Blázquez, Gutiérrez-Solana, Fernández-Moreira, Briones, Andreu, Garesse, Campos, and Arenas. Drafting of the manuscript: Martín, Blázquez, Gutiérrez-Solana, Fernández-Moreira, and Arenas. Critical revision of the manuscript for important intellectual content: Martín, Blázquez, Briones, Andreu, Garesse, Campos, and Arenas.

Obtained funding: Martín and Arenas. Study supervision: Martín and Arenas.

Funding/Support: This study was supported by grants FIS 01/1426 and FIS PI030224 from the Fondo de Investigación Sanitaria, Ministerio de Sanidad y Consumo, Madrid, and by grant FIS G03/011 from the Spanish Mitochondrial Diseases Network, Coordinación Center in Madrid. Mr Blázquez was supported by post-Médico Interno Residente contract FIS CM0300007, Mr Fernández-Moreira by grant FIS PI030224, and Dr Campos by research contract ISC III 98/3166 from the Instituto de Salud Carlos III, Madrid.

Acknowledgment: We are grateful to Pilar del Hoyo and Sara Jiménez, the technicians who worked on the respiratory chain activities.

REFERENCES
