New DGK Gene Mutations in the Hepatocerebral Form of Mitochondrial DNA Depletion Syndrome

Michelangelo Mancuso, MD; Silvio Ferraris, MD; Jacklyn Pancrudo, BS; Annette Feigenbaum, MD; Julian Raiman, MD; John Christodoulou, MBBS, FRACP, PhD; David R. Thorburn, PhD; Salvatore DiMauro, MD

Objective: To document novel homozygous mutations in the gene for deoxyguanosine kinase (DGK) in 3 children with mitochondrial DNA depletion.

Design: Clinical features included liver failure, hypotonia, and nystagmus in 2 siblings, and liver cirrhosis, optic dysplasia, nystagmus, and microcephaly in the third patient. We sequenced the whole coding region of the DGK gene.

RESULTS: We identified 2 novel homozygous mutations, G352A and C269T, that lead to truncated proteins.

Conclusion: These data confirm that DGK mutations typically affect the liver and brain.

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Subject Affiliations: Department of Neurology, Columbia University College of Physicians and Surgeons, New York, NY (Drs Mancuso, Ferraris, and DiMauro and Ms Pancrudo); Department of Neurosciences, University of Pisa, Pisa, Italy (Dr Mancuso); Division of Clinical Genetics and Metabolism, The Hospital for Sick Children and University of Toronto, Toronto, Ontario (Drs Feigenbaum and Raiman); and Murdoch Children's Research Institute, Genetic Health Services Victoria, Royal Children's Hospital, and Department of Paediatrics, University of Melbourne, Melbourne, Victoria, Australia (Drs Christodoulou and Thorburn).
A real-time quantitative polymerase chain reaction (PCR) was used to evaluate the mtDNA content in liver and muscle specimens. The entire coding region of the DGK gene was amplified and sequenced directly. The presence of the DGK mutations was confirmed by PCR–restriction fragment length polymorphism analysis. For the C269T mutation, the DNA was amplified using the following primers: forward, 5'-CTCCTICAGCGCTTATTAGG-3'; and reverse, 5'-GATTATCGACCACTGCTGC-3'. The PCR conditions were 94°C for 3 minutes, followed by 35 cycles of 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute, and a final extension step at 72°C for 7 minutes. Aliquots of PCR products were digested with BstNI restriction endonuclease and electrophoresed in 2% agarose gel.

For the G352A mutation, DNA was amplified using the following primers: forward, 5'-GTACCCCATGGAGTAAATAT-3'; and reverse, 5'-AAACAGGCAGCAGCTGACAT-3'. The PCR conditions were 94°C for 3 minutes, followed by 35 cycles of 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute, and a final extension step at 72°C for 7 minutes. Aliquots of PCR products were digested with Avall restriction endonuclease and electrophoresed in 2% agarose gel.

### MOLECULAR ANALYSES

**Table. Biochemical Analysis of Respiratory Chain Enzymes in Tissues From Patients**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Tissue</th>
<th>Complex*</th>
<th>I</th>
<th>I + II</th>
<th>II</th>
<th>II + III</th>
<th>III</th>
<th>IV</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Liver</td>
<td>15</td>
<td>NP</td>
<td>NP</td>
<td>37</td>
<td>146</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Muscle</td>
<td>42</td>
<td>NP</td>
<td>51</td>
<td>64</td>
<td>75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Liver</td>
<td>16</td>
<td>21</td>
<td>8</td>
<td>6</td>
<td>80</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: NP, not performed.

*Data are given as percentage of mean control values.

Patient 3 was born at 38.5 weeks’ gestation to nonconsanguineous Indian parents. At 21 hours of life, she was lethargic, had poor suck, and developed hypothermia, metabolic acidosis, and hypoglycemia (glucose level, 12.6 mg/dL [0.7 mmol/L]; normal, >45.0 mg/dL [>2.5 mmol/L]), requiring continuous glucose infusion. The acidosis resolved, and the glycemia remained fairly stable while 5.5 mg/kg per minute of glucose was infused. The acidosis resolved, and the glycemia remained consistently less than 27 mg/dL (normal, <27 mg/dL). A repeat muscle biopsy showed moderately severe dilation of the ventricles but no parenchymal lesions. On magnetic resonance spectroscopy, a lactate peak was observed, although her plasma lactate level was normal, <2.4 mmol/L (normal, <2.4 mmol/L), and her ammonia level was 267.5 µg/dL (normal, <85.2 µg/dL). A urinary organic acid profile showed nonspecific mild elevation. A liver biopsy specimen showed severe cholestasis, microvesicular and macrovesicular steatosis, hepatocellular dropout with nesting and pseudocinar formation, hepatocellular spotty necrosis and giant cell transformation, periporal fibrosis, and ultrastructural evidence of excessive and abnormal mitochondrial fragments.

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At the age of 1 month, she underwent liver transplantation. Postoperatively, she developed renal failure, anasarca, hypoaalbuminemia (albumin level, <2.5 g/dL; normal, 3.2-4.8 g/dL), an increasing bilirubin level, and evidence of probable sepsis, with intermittent thrombocytopenia and coagulopathy. On day 35 posttransplantation, she developed roving eye movements with nystagmus. A new magnetic resonance imaging of the brain showed moderately severe dilatation of the ventricles but no parenchymal lesions. On magnetic resonance spectroscopy, a lactate peak was observed, although her plasma lactate level was consistently less than 27 mg/dL (<3 mmol/L). A repeat muscle biopsy revealed lipid storage and rare cytochrome-c oxidase–negative fibers, but no ragged red fibers. She died at the age of 3 months (2 months posttransplantation) after developing pulmonary hypertension, pulmonary edema, and shock.

### RESULTS

A real-time PCR of liver biopsy specimens showed severe reduction of the mtDNA–nuclear DNA ratios, with 84% depletion in patient 1 and 90% depletion in patient 3. Patients 1 and 2, who were siblings, had a homozygous G→T change at nucleotide 269 (Figure A). The mutation produces a frameshift and a premature TGA stop at codon 79, resulting in the loss of 198 amino acids. Both parents were heterozygous for the mutation. Patient 3 had a homozygous G→A change at nucleotide 352 (Figure B). The mutation produces a frameshift and a premature TGA stop at codon 107, resulting in a truncated protein missing 170 amino acids. The presence of the mutation was confirmed in both families by PCR–restriction fragment length polymorphism analysis (Figure C and D). Both mutations were absent in 90 healthy control subjects.

### COMMENT

The clinical spectrum of mtDNA depletion syndrome is diverse: in some patients, only one organ is affected, while in others, the syndrome is multisystemic. The liver seems particularly vulnerable to DGK mutations, because all described patients shared severe hepatopathy as a common clinical feature. However, other organs are not spared, as our patients illustrate. Although all 3 developed liver failure and metabolic acidosis in early infancy, patient 1 also had cerebral atrophy and nystagmus; patient 2 had microcephaly, hypotonia, and nystagmus; and patient 3 had optic dysplasia with nystagmus and an abnormal second-skeletal muscle biopsy result.

It has been documented that DGK mutations cause nucleotide pool imbalance, which leads to inefficient mtDNA replication and, hence, to mtDNA depletion. All our patients had frameshift DGK mutations that resulted in truncated polypeptides. In patients 1 and 2, the premature stop codon abolishes the last 198 amino acids, whereas
in patient 3, the predicted protein is only 107 amino acids long. In both cases, the α-9 α-helical domain of the protein is lacking, virtually eliminating enzymatic activity.12 Our data seem to confirm that liver transplantation is an option only for those patients with organ-specific mtDNA depletion, as previously suggested.4,13 In patient 3, who developed multisystem disease, liver transplantation did not prevent or ameliorate brain dysfunction, as also reported in a similarly complex previous case.4 Therefore, careful screening of potential organ recipients is crucial because systemic involvement portends poor long-term prognosis.

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Correspondence: Salvatore DiMauro, MD, Room 4-420 Columbia University College of Physicians and Surgeons, 630 W 168th St, New York, NY 10032 (sd12@columbia.edu).

Author Contributions: Study concept and design: DiMauro. Acquisition of data: Mancuso, Pancrudo, Feigenbaum, Raiman, Christodoulou, and Thorburn. Analysis and interpretation of data: Mancuso, Ferraris, Pancrudo, and DiMauro. Drafting of the manuscript: Mancuso, Ferraris, Christodoulou, and DiMauro. Critical revision of the manuscript for important intellectual content: Ferraris, Feigenbaum, Christodoulou, and Thorburn. Obtained funding: DiMauro. Administrative, technical, and material support: Ferraris, Pancrudo, and Thorburn. Study supervision: Mancuso, Thorburn, and DiMauro.

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REFERENCES


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