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.

**METHODS**

**PATIENTS**

Patient 1, the first child of consanguineous Lebanese parents, was born at 38 weeks following an uncomplicated pregnancy. At birth, the child weighed 2150 g. In the immediate neonatal period, she developed hypothermia, hypoglycemia, and poor feeding. She was discharged at the age of 6 weeks, but was admitted to the hospital 2 weeks later because of poor feeding, vomiting, increased stool frequency, and poor weight gain. She had jaundice, hepatosplenomegaly, and nystagmus. Abdominal ultrasonography revealed ascites, and blood testing showed worsening of previously abnormal liver function test results, including conjugated hyperbilirubinemia, coagulopathy, and fasting hypoglycemia. A hepatobiliary scan showed marked cholestasis with preserved hepatic extraction. When she was readmitted at the age of 4 months because of an upper respiratory tract infection, liver function had further worsened and she had failure to thrive. A magnetic resonance image of the brain showed mild cerebral atrophy. She continued to deteriorate, became cachectic, developed an encephalopathy, and died at the age of 5 months.

Her venous blood lactate level was persistently elevated (range, 35.1-45.0 mg/dL [3.9-5.0 mmol/L]; normal, <18.0 mg/dL [<2.0 mmol/L]), and her liver enzyme levels were increased. A metabolic screen of the urine showed generalized amino aciduria and lactic aciduria.

A liver biopsy specimen revealed severe disruption of the normal architecture, with micronodular cirrhosis and marked cholestasis. The result of a muscle biopsy was normal. Oxidative enzyme stains and respiratory chain activities were normal in muscle, but activities of respiratory chain complexes containing mtDNA-encoded subunits (complexes I and IV) were decreased in the liver (Table). Patient 2, a younger sister of patient 1, was born at 39 weeks and weighed 2200 g. She also had poor feeding and recurrent vomiting, and developed progressive liver disease. Her plasma lactate level at the age of 2 months was 43.2 mg/dL (4.8 mmol/L). When she was admitted to the hospital for bilateral herniorrhaphies at the age of 4 months, she was hypotonic and microcephalic, with disconjugate eye movements. She also had severe failure to thrive and hepatosplenomegaly. A muscle biopsy specimen showed scattered subsarcolemmal aggregates of mitochondria. Cytochrome-c oxidase staining and respiratory chain activities were normal in muscle, but a liver sample was not available for analysis (Table). She died at the age of 4 months of hepatic failure.
Increased to 1.16 administered. During the next 4 days, her total bilirubin level remained fairly stable while 5.5 mg/kg per minute of glucose was cose infusion. The acidosis resolved, and the glycemia re-

Molecular Analyses

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’-CTCCTICACCCCTGATTGGG-3’; and reverse, 5’-ATTATCTCCACCTGCTGC-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’-GTACCCCATGAGAATAAT-3’; and reverse, 5’-AAAAAAGGCGACTGAGCAT-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 AvaI restriction endonuclease and electrophoresed in 2% agarose gel.

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.4,6 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.6 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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Figure. Electrophoretograms (A and B) and polymerase chain reaction–restriction fragment length polymorphism analysis (C and D) of the 2 mutations. A, C→T at nucleotide 269 (underlined) from patients 1 and 2 (sequence of the complementary strand). B, G→A at nucleotide 352 (underlined) from patient 3 (sequence of the complementary strand, as in panel A). C, Wild-type DNA is cut into fragments by the BstNI restriction enzyme, while mutant DNA is not. D, Wild-type DNA is cut into fragments by the AvaI restriction enzyme, while mutant DNA is not. M indicates molecular marker; P, patient; C, healthy control; and U, undigested DNA.