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

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Objective: To document novel homozygous mutations in the gene for deoxynucleosine 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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Mitocondrial DNA (mtDNA) depletion syndrome encompasses a clinically heterogeneous group of disorders sharing marked reduction of mtDNA copy number in one or more tissues. Primary mtDNA depletion syndrome is transmitted as an autosomal recessive trait and can affect single organs, typically the muscle or liver, or multiple tissues. Mutations in the deoxynucleosine kinase (DGK) gene usually result in the hepatic form of mtDNA depletion syndrome, whereas mutations in the thymidine kinase 2 (TK2) gene are found in patients with the myopathic form.

Herein, we document mtDNA depletion in liver due to novel homozygous DGK mutations in 2 unrelated families with hepatocerebral syndromes.

METHODS

PATIENTS

Patient 1, the first child of consanguineous Lebanese parents, was born at 38 weeks following an uncomplicated pregnancy. The birth weight was 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, hepatomegaly, 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 cerebrocerebral 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 mitochondrial 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 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 mitochondrial complexes containing mtDNA-encoded subunits (complexes I and IV) were decreased in the liver (Table).
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 3.5 mg/kg per minute of glucose was administered. During the next 4 days, her total bilirubin level increased to 1.16 mg/dL (1.98 × 10^6 μmol/L) (conjugated bilirubin level, <1 mg/dL [<17 µmol/L]), her aspartate aminotransferase level was 136 IU/L (normal, <110 IU/L), her alanine aminotransferase level was 73 IU/L (normal, <60 IU/L), her lactate level was 84.7 mg/dL (9.4 mmol/L) (normal, <21.6 mg/dL [<2.4 mmol/L]), and her ammonia level was 267.5 µg/dL (157 µmol/L) (normal, <85.2 µg/dL [<50 µmol/L]). She progressed to liver failure, with cholestasis, hypoaalbuminemia, portal hypertension with intractable ascites, hypersplenism with intermittent thrombocytopenia, a prolonged international normalized ratio, and an elevated partial thromboplastin time.

She was small, but physical and neurological examination results were normal. A formal ophthalmologic examination showed mild optic dysplasia and an immature retina. Brainstem auditory- and visual-evoked responses were normal. The electroencephalographic result was mildly abnormal, showing occasional positive rolandic sharp waves. The result of brain magnetic resonance imaging was normal. The electrocardiogram and echocardiogram, and renal function, were normal. A urinary organic acid profile showed nonspecific mild elevation of multiple dicarboxylic acids.

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, perportal fibrosis, and ultrastructural evidence of excessive and abnormal mitochondrial proliferation. A muscle biopsy specimen showed mild nonspecific abnormalities. Activities of respiratory chain complexes containing mtDNA-encoded subunits I, II plus III, and IIV were decreased in the liver (Table).

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 image of the brain 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 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.

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

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Tissue</th>
<th>Complex*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Liver</td>
<td>I + II</td>
</tr>
<tr>
<td>2</td>
<td>Muscle</td>
<td>II + III</td>
</tr>
<tr>
<td>3</td>
<td>Liver</td>
<td>I + II</td>
</tr>
</tbody>
</table>

Abbreviation: NP, not performed.

*Data are given as percentage of mean control values.

### RESULTS

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′-CTCCTCTACCGCTGGATTGAC-3′; and reverse, 5′-GATTATGCAATGCGCTG-3′. The PCR results were normal, 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′-GTACCCCATGGGAGTAAATAT-3′; and reverse, 5′-AAACAGGCGAGCCTAGCAT-3′. The PCR results 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 AvaII restriction endonuclease and electrophoresed in 2% agarose gel.

### MOLECULAR ANALYSES

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. 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-skelletal 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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REFERENCES