Hepatocerebral Mitochondrial DNA Depletion Syndrome Caused by Deoxyguanosine Kinase (DGUOK) Mutations

Peter Freisinger, MD; Nancy Fütterer, MD; Erwin Lankes, MD; Klaus Gempel, MD; Thomas M. Berger, MD; Johannes Spalinger, MD; Alexandra Hoerbe, MD; Claudia Schwantes, MD; Martin Lindner, MD; René Santer, MD; Martin Burdelski, MD; Hansjörg Schaefer, MD; Bernhard Setzer, PhD; Ulrich A. Walker, MD; Rita Horváth, MD

Background: Autosomal recessive mutations in deoxyguanosine kinase (DGUOK) have been identified in the hepatocerebral form of mitochondrial DNA (mtDNA) depletion syndrome.

Objectives: To describe the clinical spectrum of DGUOK-related mtDNA depletion syndrome in 6 children and to summarize the literature.

Results: We identified pathogenic mutations in DGUOK in 6 children with the hepatocerebral form of mtDNA depletion syndrome. We describe the clinical, neuroradiologic, histologic, and genetic features in these children. All children showed severe hepatopathy, while involvement of other organs (skeletal muscle and brain) was variable. We identified 5 novel mutations (1 of them in 2 children) and 2 previously described mutations. Three different mutations affected the initial methionine, suggesting a mutational hot spot. One of our patients underwent liver transplantation; pathologic findings revealed (in addition to diffuse hepatopathy) a hepatocellular carcinoma, implying a possible link between mtDNA depletion syndrome and tumorigenesis.

Conclusion: We studied 12 children with infantile hepatencephalopathies and mtDNA depletion syndrome and found pathogenic DGUOK mutations in 6, suggesting that this gene defect is a frequent but not an exclusive cause of the hepatic form of mtDNA depletion syndrome.

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Mitochondrial DNA (mtDNA) depletion syndrome is defined as a reduction in mtDNA copy number in different tissues, leading to insufficient synthesis of respiratory chain complexes I, III, IV, and V.1-4 Autosomal recessive mutations of 5 nuclear genes have been identified in patients with mtDNA depletion syndrome with different clinical presentations.5 Onset of symptoms usually is in the first year of life, and most patients die in early childhood.6 Thymidine phosphorylase (TP) defects are associated with leukodystrophy and gastrointestinal disorder.7 Mutations in deoxyguanosine kinase (DGUOK)8-18 and polymerase γ (POLG)19-22 have been described in the hepatocerebral form of mtDNA depletion syndrome, whereas thymidine kinase 2 (TK2) mutations are associated with myopathy and an elevation in the level of creatine kinase.7,8,9 Recently, mutations in succinyl coenzyme A synthase (SUCLA2) were identified in 4 related infants with severe psychomotor retardation, epilepsy, hearing loss, anemia, and Leigh-like lesions on magnetic resonance imaging.20 Mitochondrial depletion can initially affect single organs, typically skeletal muscle or liver, and subsequently spread to other tissues.4,6

Herein, we describe the clinical, histologic, and genetic findings in 6 children carrying pathogenic mutations in DGUOK. Five of these mutations have not been reported before, to our knowledge.

METHODS

PATIENT RECRUITMENT

In the past 15 years, we recruited 141 pediatric patients (<15 years) with combined respiratory chain enzyme deficiency. Hepatopathy was noted in 39 patients, who were further investigated using molecular genetic studies. Twelve of these children showed mtDNA depletion syndrome. Sequencing of DGUOK revealed pathogenic mutations in 6 patients (Table 1). Patient 1

This girl was the first child of nonconsanguineous parents of Polish origin. At age 2 months, she developed cholestasis, feeding difficul-
ties, failure to thrive, and coagulation problems. Laboratory screening at age 2 months showed a high serum tyrosine level. Urinary amino acid assay showed a generalized hyperaminoaciduria. Coagulation test results and liver enzyme levels were abnormal. Serum bile acid levels were increased, and the α-lipoprotein level was elevated (3242.2 ng/mL; normal level, <7 ng/mL). Ammonia levels were never elevated. Hepatobiliary scintigraphy revealed parenchymal liver damage and an intraparenchymal block of bile excretion. A liver biopsy was carried out at the age of 2½ months. Cardiologic examination showed impaired left bundle conduction and persistent foramen ovale and ductus arteriosus. Brain magnetic resonance imaging was normal.

Between 2 and 11 months of age, liver function continued to deteriorate, and the patient required regular fresh frozen plasma transfusions. Abdominal ultrasonography revealed a solid tumor in the liver. The serum α-lipoprotein level was increased (39 634.9 ng/mL). The patient had no sign of neurologic impairment or involvement of other tissues. She underwent liver transplantation at 1 year of age, and pathologic examination of the liver showed (in addition to the expected diffuse degenerative hepatopathy) a localized hepatocellular carcinoma.

Patient 2

This boy was the second child of distantly related German parents. One older sister is healthy. At age 7 weeks, he had jaundice and weakness of trunk, neck, and arm muscles. He could not fix his gaze and had constant striking horizontal nystagmus. There was no palpable hepatosplenomegaly. Laboratory test results revealed serum lactate levels up to 72 mg/dL (reference level, <18 mg/dL), elevated transaminase levels, and consistent preprandial hypoglycemia. Cerebrospinal fluid lactate and protein levels were also increased. Echocardiography was normal for age. Liver, skin, and skeletal muscle biopsy specimens were obtained. He developed severe feeding difficulties, became weak, and required percutaneous gastrostomy at age 12 months. Brain magnetic resonance imaging at age 1 year showed no significant abnormalities; a solid tumor was noted on abdominal ultrasonography. At this time, the serum α-lipoprotein level was increased (10 961.5 ng/mL).

Patient 3

This girl, the second child of nonconsanguineous Russian parents, was born after a normal pregnancy and delivery. A similarly affected sister died at the age of 5 months. The first symptoms of the index patient at the age of 2 weeks were hypoglycemia, feeding difficulties, and failure to thrive followed by coagulopathy, hepatopathy with prolonged jaundice, and muscular hypotonia at the age of 4 weeks. Laboratory screening showed high levels of tyrosine, serum lactate, bilirubin, and γ-glutamyltransferase. A muscle biopsy specimen was obtained. The hepatopathy progressed rapidly, leading to death at age 13 months.

Patient 4 and Patient 5

Patient 4 was the second child of nonconsanguineous Portuguese parents. Their first child is healthy. Pregnancy and delivery were normal. At the age of 3 days, patient 4 (a boy) developed generalized hypotonia, tachypnea, lactic acidosis, hypoglycemia, and severe coagulopathy. This acute episode was corrected in an intensive care unit, but during the following months he developed progressive hepatopathy, hypotonia, nystagmus, and psychomotor retardation. Brain magnetic resonance spectroscopy revealed increased lactate peaks. Hepatic dysfunction progressed to end-stage liver failure and death at the age of 9 months.

The initial presentation of patient 5, a similarly affected younger sister of patient 4, included lactic acidosis, hypoglycemia, and coagulopathy noted on the third day of life. Her condition stabilized, and she was discharged home after 4 weeks. At age 4 months, she was growing well and had moderate cho-
llestasis with slightly elevated transaminase levels. At age 5 months, she does not have lactic acidosis or hypoglycemia.

**Patient 6**

This girl was the third child of nonconsanguineous Turkish parents with no family history of neuromuscular disease. Pregnancy and delivery were normal. Hypotonia was noted at age 3 months. At age 4 months, she was examined because of repeated vomiting, failure to thrive, and lactic acidosis (50.4-58.5 mg/dL). On neurologic examination, she had nystagmus, generalized muscular hypotonia, and severe bilateral sensorineural hearing loss. Her feeding problems worsened, and additional signs of hepatopathy were detected. The child is alive at age 2 years.

**MORPHOLOGIC STRUCTURE AND BIOCHEMICAL ANALYSIS OF SKELETAL MUSCLE AND LIVER**

Six-micrometer serial cross sections of muscle biopsy specimens were obtained for histochemical staining according to standard procedures. A frozen portion of the biopsy specimen was used for biochemical analysis. Activities of respiratory chain complexes I through IV were determined in skeletal muscle as previously described.

**DNA ANALYSIS**

DNA extraction from muscle, liver, and blood was performed according to standard purification protocols (Qiagen, Hilden, Germany). Mitochondrial DNA and nuclear DNA (nDNA) copy numbers within tissues were determined by quantitative polymerase chain reaction using the ABI 7700 sequence detection system (Applied Biosystems, Foster City, Calif) as previously described. All samples were run in triplicate. Absolute mtDNA and nDNA copy numbers were calculated using serial dilutions of plasmids with known copy numbers. DGUOK was sequenced as previously described. For complementary DNA analysis, we used the following primers: 5'-CTCTTTCAAGTCGGGCTTCG-3' (forward primer) and 5'-CTTTACAAAGGTGTTTACCT-3' (reverse primer). Both initial methionine mutations were tested using a restriction digest with BciI on the polymerase chain reaction product of exon 1. Restriction fragment length polymorphism analysis for S32F was performed using a forward mismatch primer: 5’-ATTTCAGTCGCTGGAGAG-3’ together with the reverse primer of exon 2α and digested with MlyI. Restriction fragment length polymorphism analysis for Q170R was performed using the forward mismatch primer 5’-AGTGACATCGAGAGCATCTAG-3’ and the reverse primer of exon 4α and digested with FauI.

**RESULTS**

**MORPHOLOGIC STRUCTURE AND BIOCHEMICAL ANALYSIS OF SKELETAL MUSCLE AND LIVER**

In patient 1, histologic examination of the liver showed siderosis, tubular transformation of hepatocytes, and extracellular, canalicular, and hepatocellular cholestasis (Figure). On electron microscopy, the hepatocytes showed enlarged mitochondria with reduction of cristae. Histologic examination of the explanted liver after transplantation identified a localized hepatocellular carcinoma.

In patient 2, histologic examination of the liver showed steatosis, siderosis, and canalicular and hepatocellular cholestasis. Electron microscopy demonstrated accumulation of mitochondria with reduction of cristae. Histologic examination of muscle biopsy specimens was normal. Respiratory chain enzyme activity in muscle was normal.

In patient 3, skeletal muscle biopsy revealed normal histologic findings. However, activities of the respiratory chain complexes were decreased.

Patients 4 and 5 underwent liver biopsy (at age 10 weeks and 18 days, respectively). In patient 4, histologic examination showed neonatal giant cell hepatitis with multinucleated giant cells, intrahepatic cholestasis, and localized steatosis. In patient 5, there was marked cholestasis, mild siderosis, and mild portal inflammation but no multinucleated giant cells. Results of viral studies (cytomegalovirus, Epstein-Barr virus, hepatitis B virus, and herpes simplex virus) were negative.

In patient 6, histologic examination of a skeletal muscle biopsy specimen at 1 year of age showed scattered atrophic fibers of both types (ie, types I and II), increased mitochondrial accumulation in several fibers, scattered succinate dehydrogenase hyperreactive and cytochrome-c oxidase–negative ragged red fibers, and lipid accumulation in ragged red fibers. Biochemically, there was severe combined reduction of complex I, III, and IV activities. No liver biopsy was performed.

**DNA ANALYSIS**

Real-time polymerase chain reaction showed mtDNA depletion in liver DNA of patients 1, 2, and 5 (Table 1). In patient 6, muscle mtDNA was depleted. In patients 3 and 4 no liver or muscle tissue was available for mtDNA depletion studies.
MUTATIONS REVEALED BY SEQUENCING OF DGUOK

Patient 1 was compound heterozygous for 2 missense mutations, M1V and M11. Both mutations affect the first methionine of the protein. In patient 2, we found an S52F homozygous missense mutation affecting a well-conserved amino acid (Table 2). The mutation was not present in 50 control samples. The same mutation was present heterozygously in patient 3. The other allele of patient 3 carried a 4–base pair (bp) GTTT deletion in exon 5, leading to a frameshift. Patients 4 and 5 were compound heterozygous for 2 previously described pathogenic missense mutations, L250S in exon 6 and M1T in exon 1, affecting the initial methionine of the protein. In patient 6, we detected a heterozygous missense mutation in exon 4, Q170R, which changes a conserved amino acid (Table 2) and was not present in 50 control samples. The father of this child was heterozygous for Q170R, but the mother was negative for this mutation. Sequencing the exon-intron boundaries in the patient and mother did not detect a second mutation. Analysis of DGUOK cDNA from muscle of the patient revealed 2 bands, a band of normal-length cDNA harboring the Q170R mutation and a smaller band (approximately 260 bp, detected by real-time polymerase chain reaction) representing a short transcript consisting of exon 1, exon 6, and exon 7 but missing exons 2 through 5. However, this short transcript was also detected in control cDNA, suggesting that this band does not result from a splicing defect. Further studies are in progress to identify a second pathogenic mutation in this family.

The first DGUOK mutation associated with the hepato-cerebral form of mtDNA depletion syndrome was reported in 2001. Since then, 40 cases from 21 families have demonstrated 14 different pathogenic DGUOK mutations (Table 3). Six were frameshift or nonsense mutations, and 8 were missense mutations. We identified 5 novel (Table 1) and 2 previously described DGUOK mutations. Six were missense mutations affecting conserved amino acids, and 1 mutation led to a frameshift and premature termination. Three different mutations altered the initial methionine of the protein, as recently reported by others. Further studies will test whether the loss of the normal initial methionine results in reduced levels of normal proteins or in abnormal or shortened proteins. In patient 3, we detected a 4-bp GTTT deletion causing a frameshift and premature termination. The S52F missense mutation affects a well-conserved amino acid (Table 2) and was not present in 50 control samples. In patient 3, S52F was present in compound heterozygous form together with the frameshift mutation, while in patient 2 (from another consanguineous family) it was homozygous. In patient 6, only 1 heterozygous missense mutation was detected. The Q170R mutation is situated in a well-conserved region (Table 2), close to the active site of the enzyme that is responsible for the binding of all substrates, and was not detected in 50 control samples.

A review of the clinical presentations of our patients and those of previously described patients suggests that children carrying missense mutations have later onset of symptoms and slower progression of the disease. Some of these patients initially demonstrated isolated hepato-pathy, without clinical or neuroradiologic signs of central nervous system involvement, although most patients developed neurologic symptoms at a later stage of the disease.

Severe hepatic involvement in association with mtDNA depletion syndrome has been described in patients carrying mutations in DGUOK or POLG1. In children with Alpers syndrome and POLG1 mutations, the most prominent symptom is intractable progressive epilepsy. Liver involvement can be mild initially but may worsen rapidly, especially after exposure to valproic acid. In DGUOK deficiency, liver involvement seems to be the most prominent feature, leading to liver cirrhosis and causing early-onset liver failure. In contrast, the encephalopathy may be mild or absent. Neuropathologic examination typically shows white matter degeneration in the cerebral and cerebellar hemispheres and mild astrocytosis. Liver failure may contribute, at least in part, to the encephalopathy.

In patient 2, histologic findings of muscle biopsy specimens and respiratory chain activities were normal, as reported in 7 previously published cases. This finding emphasizes the importance of investigating the most affected tissue in these patients because the mitochondrial etiology of the disease might be overlooked by studies confined to skeletal muscle. On the other hand, prominent muscle involvement with mtDNA depletion syndrome does not exclude a defect in DGUOK, as illustrated by patient 6. It is unclear why some patients show isolated hepatic involvement and others develop multisystem disease, but this might be related to the various pathogenic effects of different mutations.

Our patients with DGUOK defects had no evidence of epilepsy. Seizures were described in only 1 previously published case during the terminal stage of the disease, complicated by shock, hematemesis, and hypoglycemia. Previous electroencephalographic examination...

| Table 2. Conservation of the Missense Mutations S52F and Q170R |
|-----------------|-----------------|
| Genus Species   | Missense Mutation |
| Conservation of S52F |                  |
| Homo sapiens    | NIAVGKSTFKLTT    |
| Bos taurus      | NIAVGKSTFKLTT    |
| Canis familiaris| NIAVGKSTFKLTT    |
| Mus musculus    | NIAVGKSTFKLMLM   |
| Xenopus         | NIAVGKSTFRLLLL   |
| Tetraodon       | NIAVGKSTFVLLE    |
| Conservation of Q170R |
| Homo sapiens    | DIEWHYQDWHSFLL   |
| Bos taurus      | DIEWHYQDWHSFLL   |
| Canis familiaris| DIEWHYQDWHSFLL   |
| Mus musculus    | DIEWHYQDWHSFLL   |
| Xenopus         | MEWTLQDEWHFLL    |
| Tetraodon       | PTEWEAIVQDWHSLL  |

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tion of this child had not shown any abnormalities. In general, seizures are unusual in children with DGUOK mutations and may result from metabolic crises caused by end-stage hepatopathy. Most patients had prominent ocular movement disturbances, including oscillating and disconjugated eye movements and rotatory, pendular, or multidirectional nystagmus. Nystagmus was absent only in a few cases, and these children had a generally milder clinical course. Nystagmus was the first sign of neurological involvement in 4 of our 6 patients. Conversely, the absence of eye abnormalities might presage a better prognosis and a possible beneficial effect of liver transplantation.

Liver transplantation was performed in 6 previously described patients. Four of these had multisystem presentation at the time of the transplantation (which did not stop progression of the disease in different organs) and subsequently died. In contrast, the other 2 patients, who carried missense mutations in DGUOK, demonstrated isolated hepatopathy, and underwent liver transplantation at 12 and 17 months of age, had no involvement of other organs at 3 and 5 years of age. The child described by Salviati et al is 11 years old and, except for episodic migraine, has no involvement of other tissues (Annette Feigenbaum, MD, written communication, December 2005). Patient 1 in our study underwent liver transplantation. Unexpectedly, a localized hepatocellular carcinoma was found in addition to the diffuse degenerative hepatopathy, implying a possible relationship between the mitochondrial defect and carcinogenesis. The association of DGUOK mutations with tumors has not been described, to our knowledge. Mitochondria have been implicated in carcinogenesis because of their role in apoptosis. Scheers et al recently described 2 children with isolated hepatopathy and combined respiratory chain deficiency who developed hepatocellular carcinoma in later stages of their disease. Mitochondrial DNA depletion and deletions were excluded in these cases. Achanta et al showed evidence that hepatocellular mtDNA depletion may lead to loss of the tumor suppressor p53 protein and to increased risk of tumorigenesis in the liver. Whether the observed mtDNA damage has a primary and causative link in the process of cancer development or whether it may represent a secondary bystander effect needs to be investigated. α-Fetoprotein is a sensitive marker but not a specific marker that is used to differentiate hepatocellular carcinoma from nonmalignant liver disease. Three previous reports of patients with DGUOK mutations mentioned elevation of α-fetoprotein levels. Liver transplantation was performed in 2 of these patients, but no hepatocellular carcinoma was reported. Although the diagnostically valuable information of an elevated α-fetoprotein level in the detection of hepatocellular carcinoma in DGUOK deficiency is not clear, the possibility of an additional hepatocellular carcinoma should be considered in patients with

### Table 3. Summary of Previously Described Mutations in DGUOK

<table>
<thead>
<tr>
<th>Exon</th>
<th>Mutation</th>
<th>Effect</th>
<th>Central Nervous System Involvement</th>
<th>Muscle Involvement</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>c.272→C homozygous</td>
<td>M1T</td>
<td>Nystagmus, failure to thrive</td>
<td>Hypotonia RC ↓</td>
<td>Slama et al, 2005</td>
</tr>
<tr>
<td>2</td>
<td>c.204delA homozygous</td>
<td>Frameshift, stop after aa 80</td>
<td>Nystagmus</td>
<td>Hypotonia, RC ↓</td>
<td>Slama et al, 2005</td>
</tr>
<tr>
<td>3</td>
<td>c.235C&gt;T homozygous</td>
<td>Stop after aa 79</td>
<td>Nystagmus</td>
<td>Normal</td>
<td>Mancuso et al, 2005</td>
</tr>
<tr>
<td>4</td>
<td>c.313C&gt;T homozygous</td>
<td>Stop after aa 105</td>
<td>Nystagmus, encephalopathy</td>
<td>Normal</td>
<td>Taarnman et al, 2002</td>
</tr>
<tr>
<td>5</td>
<td>c.318G&gt;A homozygous</td>
<td>Stop after aa 106</td>
<td>Optic dysplasia, nystagmus</td>
<td>Normal</td>
<td>Mancuso et al, 2005</td>
</tr>
<tr>
<td>4</td>
<td>c.494A&gt;T, c.797T&gt;G</td>
<td>E165V, L266R</td>
<td>Developmental delay</td>
<td>Hypotonia, RC ↓</td>
<td>Slama et al, 2005</td>
</tr>
<tr>
<td>5, 6</td>
<td>c.632A&gt;G, c.797T&gt;G</td>
<td>E211G, L266R</td>
<td>Developmental delay</td>
<td>Hypotonia, RC ↓</td>
<td>Slama et al, 2005</td>
</tr>
<tr>
<td>4</td>
<td>c.609-610delGT homozygous</td>
<td>Frameshift, stop after aa 213</td>
<td></td>
<td></td>
<td>Salviati et al, 2002</td>
</tr>
<tr>
<td>6</td>
<td>c.709T&gt;C homozygous</td>
<td>L250S</td>
<td>Nystagmus, dystrophy</td>
<td>Hypotonia RC ↓</td>
<td>Wang et al, 2005</td>
</tr>
<tr>
<td>3</td>
<td>c.783G&gt;T homozygous</td>
<td>D253Y</td>
<td>Cystathioninuria?</td>
<td>RC ↓</td>
<td>Tadiboyina et al, 2005</td>
</tr>
<tr>
<td>6</td>
<td>c.766-768insGATT homozygous</td>
<td>Frameshift, stop after aa 255</td>
<td>Nystagmus, developmental delay</td>
<td>Hypotonia RC ↓</td>
<td>Salviati et al, 2002</td>
</tr>
<tr>
<td>6</td>
<td>c.766-768insGATT homozygous</td>
<td>Frameshift, stop after aa 255</td>
<td>Nystagmus, abnormal visual evoked potential, brainstem auditory evoked potential</td>
<td>Hypotonia RC ↓</td>
<td>Rabinowitz et al, 2004</td>
</tr>
<tr>
<td>6</td>
<td>c.766-768insGATT homozygous</td>
<td>Frameshift, stop after aa 255</td>
<td>Nystagmus, psychomotor delay, pyramidal signs</td>
<td>Hypotonia, failure to thrive</td>
<td>Labarthe et al, 2005</td>
</tr>
</tbody>
</table>

Abbreviations: aa, amino acid; del, deletion; ins, insertion; RC, respiratory chain activity.
solid tumor detected by abdominal ultrasonography and increased α-fetoprotein levels.

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Author Affiliations: Metabolic Disease Center Munich-Schwabing (Drs Freisinger, Futterer, Lankes, Gempel, and Horváth); Institutes of Clinical Chemistry, Molecular Diagnostics, and Mitochondrial Genetics, Academic Hospital Schwabing (Drs Gempel and Horváth); and Children’s Hospital and Institute of Medical Genetics, Technical University Munich (Drs Freisinger, Futterer and Lankes); Munich; Pediatric Clinic, Hospital of the City of Cologne, Cologne (Drs Hoerbe and Schwantes); Pediatric Clinic, University of Heidelberg, Heidelberg (Dr Lindner); Institute of Pathology, University of Hamburg, Hamburg (Drs Santer, Burdelski, and Schaefer); and Department of Rheumatology, Freeburg University Hospital, Freiburg (Drs Setzer and Walker), Germany; and Pediatric Clinic Kinderspital Luzern, Luzern, Switzerland (Drs Berger and Spalinger).

Correspondence: Rita Horváth, MD, Metabolic Disease Center Munich-Schwabing, Institutes of Clinical Chemistry, Molecular Diagnostics, and Mitochondrial Genetics, Academic Hospital Schwabing, Kolner Platz 1, 80804 Munich, Germany (Rita.Horvath@lrz.uni-muenchen.de).


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