

## A novel mutation in the *DGUOK* gene in a Turkish newborn with mitochondrial depletion syndrome

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Deoxyguanosine kinase (*DGUOK*) catalyzes the first step of the mitochondrial deoxypurine salvage pathway, the phosphorylation of purine deoxyribonucleosides. Mutations in the *DGUOK* gene have been linked to inherited mitochondrial (mt)DNA depletion syndromes, neonatal liver failure, nystagmus, and hypotonia. We now report a novel homozygous c.34C>T (p.Arg12X) mutation found in an affected newborn of asymptomatic consanguineous parents. Respiratory distress started in the first hours after birth. The patient died at the age of 42 days due to liver failure. This genotype, which is to be expected for a homozygous stop codon mutation in exon 1, is associated with a severe clinical presentation.

**Key words:** mitochondrial DNA depletion syndrome, mtDNA, *DGUOK*, deoxyguanosine kinase (*dGK*), hepatoenkephalopathy, hypoglycemia, lactic acidosis.

Mitochondrial DNA (mtDNA) depletion syndromes (MIM 251880) are a genetically and clinically heterogeneous group of autosomal recessive diseases characterized by a reduction in mtDNA copy number in different tissues, resulting in insufficient synthesis of respiratory chain complexes I, III, IV, and V<sup>1</sup>. Mutations in nine nuclear genes are currently known to cause mtDNA depletion<sup>2,3</sup>. The main clinical presentations of mtDNA depletion include the myopathic, encephalomyopathic and hepatocerebral forms<sup>4</sup>. In the hepatocerebral form, progressive liver failure, hypoglycemia, lactic acidemia, and neurological abnormalities are often present in early life. *DGUOK* mutations are the most common cause of mitochondrial hepatoenkephalopathy, and mutations can cause variable phenotypes, ranging from isolated liver disease to multi-systemic disorders<sup>5</sup>. Onset of symptoms is usually in the first year of life, and most patients die early in childhood.

Here we describe the clinical, histologic and genetic findings in a Turkish newborn carrying a novel pathogenic homozygous mutation in the *DGUOK* gene.

### Case Report

This male newborn was the second child of a first-cousin marriage of Turkish origin. Their first born was healthy. Pregnancy and delivery were both normal. At the age of 4 hours, the patient developed poor sucking, respiratory difficulty, tachypnea, hypoglycemia, and lactic and metabolic acidosis. Clinical examination showed mild hepatomegaly, jaundice, abdominal distension and ascites. Lactic acidosis improved with bicarbonate treatment; however, he developed progressive hepatopathy, hypotonia, nystagmus, seizures, and psychomotor retardation. Hepatic dysfunction progressed to end-stage liver failure and death at 42 days of life. Laboratory screening showed mild hypoglycemia (glucose: 40 mg/dl [N: 70-110]). Coagulation tests were abnormal (international normalized ratio: 5 [0.75-1.5], activated partial thromboplastin time: 62 s [25-40 s]), and liver transaminases levels were also abnormal: ALT (alanine aminotransferase) was 53 U/L (5-45); AST (aspartate aminotransferase) was 203 U/L (20-60); GGT (gamma glutamyl transferase) was 122 U/L (0.0-40); and alkaline phosphatase

was 310 U/L (91–375). Total bilirubin was 7 mg/dl (0.0–1.0) and direct bilirubin was 4 mg/dl (0.0–0.6). Serum lactate levels were high, up to 200 mg/dl (reference level <18 mg/dl). Serum  $\alpha$ -fetoprotein levels were elevated (9983–7962 ng/ml [N: <7 ng/ml]). Blood ammonia was mildly elevated, on one occasion (138/58  $\mu$ g/dl [N: 20–120]). Ferritin was elevated (2522 mg/dl [N: 30–400]). Tandem mass spectroscopy revealed significantly raised tyrosine, alanine and glycine. Plasma amino acids showed marked elevations of tyrosine and alanine. Urine organic acid revealed multiple organic acid elevations including: lactic acid, pyruvic acid and para-hydroxy compounds. Abdominal ultrasonography revealed a diffuse ascites. Hepatobiliary scintigraphy revealed decreased hepatocellular uptake. Diagnostic evaluation showed negative serology for hepatitis viruses. Transferrin isoelectric focusing was normal. Cardiologic examination showed patent ductus arteriosus and pulmonary hypertension. Brain magnetic resonance imaging (MRI) and electroencephalography were normal. Brain MR spectroscopy revealed an increased lactate peak. Muscle biopsy microscopy results were nonspecific. Respiratory chain complexes were not studied. Liver function continued to deteriorate, and the patient required regular fresh frozen plasma transfusions. The liver failure progressed in spite of supportive therapy with ursodeoxycholic acid and vitamin K1. Postmortem liver tissue revealed severe hepatocellular damage, cholestasis and fibrosis (Fig. 1).

DNA isolated from leukocytes was used to amplify the coding exons of several genes involved in the mtDNA depletion syndromes by automated sequencing using BigDye Terminator Cycle Sequencing techniques. Sequence analysis of the exons and flanking introns of the *MPV17* and *TWINKLE* genes showed no pathogenic mutations. However, sequence analysis of the *DGUOK* gene (RefSeq NM\_080916.1) revealed a novel homozygous c.34C>T mutation in exon 1 that results in a frameshift with a premature stop codon (p.Arg12X). Both parents were heterozygous for this mutation. No liver or muscle tissue was available for mtDNA depletion studies.

## Discussion

MtDNA depletion resulting in liver failure has been associated with bi-allelic mutations

in four nuclear genes [*POLG*, *DGUOK*, *MPV17*, *C<sub>10orf2</sub>* (*TWINKLE*)]<sup>4</sup>. Identification of the specific deficiency carries important prognostic information<sup>4</sup>. The major supply of deoxynucleotides for mtDNA biosynthesis depends on salvage pathways for dNTP generation. *DGUOK* is responsible for the rate-limiting first step of the salvage biosynthesis of purine deoxynucleotides that is necessary for the maintenance of mitochondrial deoxynucleotide pools<sup>6</sup>. Patients with deoxyguanosine kinase (dGK) deficiency typically present with liver dysfunction at birth or infancy, with or without neurological impairment, and most patients die before 4 years of age of liver failure<sup>3</sup>. More than 80 affected patients from nearly 50 families with *DGUOK* mutations have been reported previously<sup>1–15</sup>. Mutations located in different parts of the protein apparently have different phenotypic effects<sup>3</sup>. We found a novel homozygous c.34C>T (p.Arg12X) mutation in our patient. To our knowledge, this mutation has not been identified previously<sup>7</sup>. Freisinger et al.<sup>1</sup> reported a c.599A>G (Q170R) mutation in a Turkish infant. There is no other reported Turkish case. This case is the first report of mtDNA depletion syndrome in the *DGUOK* gene from Turkey. Thus, we do not know whether this mutation is the common cause of mtDNA depletion in Turkey. The variable age of onset of liver disease is clinically indistinguishable in the spectrum of *DGUOK* mutations. The residual dGK activity seems to play a crucial role in disease progression<sup>16</sup>. Patients who completely lacked dGK activity suffered from an early onset of the disease and died early in infancy<sup>16</sup>.

We found hypoglycemia, coagulopathy, cholestasis, cytotoxicity (with significant elevations in ALT, AST and GGT), hyperferritinemia, and lactic acidosis with an elevated L/P ratio. In addition to signs of liver failure, hypotonia and nystagmus were also seen. Because of liver failure, hypoglycemia and hyperferritinemia neonatal hemochromatosis was considered in the differential diagnosis, but it was excluded on the ground of abdominal MRI findings. Congenital glycosylation disorders of defect were also considered as a possible diagnosis because of liver failure and coagulopathy, but transferrin isoelectric focusing did not support this diagnosis. Galactosemia, tyrosinemia type I and infections were excluded before the confirmation of the mtDNA depletion syndrome

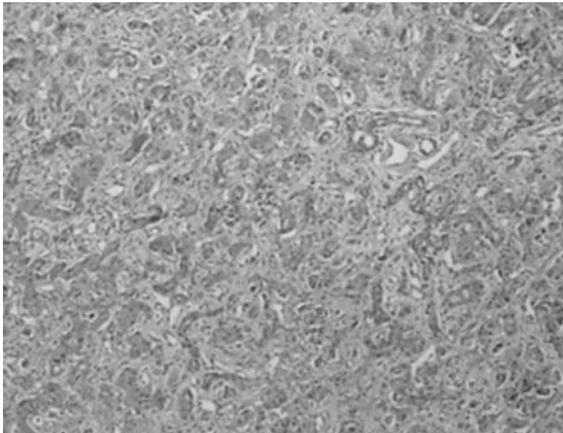


Fig. 1. Postmortem liver tissue revealed severe hepatocellular damage, cholestasis and fibrosis.

diagnosis. The presence of neurological signs in these patients is usually associated with early mortality. Elevated serum tyrosine, glycine and alanine levels were observed in our patient. Elevated tyrosine levels, a non-specific marker of hepatic dysfunction in children, can be observed in *DGUOK* patients, but are not constant in *dGK* deficiency<sup>3,4</sup>. However, it is also important to consider this diagnosis in newborns who test negative for tyrosinemia<sup>3,4</sup>. Brain MRI was normal and brain MR spectroscopy revealed increased lactate peaks. However, abnormal white matter and moderate hyperintensity in the bilateral pallidi were only seen in some patients and after 1 year of age<sup>15</sup>. Dimmock et al.<sup>13</sup> showed that the presence of neurological features is associated with poor long-term survival in patients with *DGUOK* deficiency, and liver transplantation appears futile if any of these features are present. Our patient had hypotonia, developmental delay and nystagmus; thus, liver transplantation was not considered as a treatment choice. Hepatic dysfunction progressed to end-stage liver failure and death at 42 days of life in spite of supportive treatment. Saada et al.<sup>17</sup> showed that supplementation of deoxyguanosine and deoxyadenosine normalized the mitochondrial dNTP pools and mtDNA content and partially restored the MRC function and can serve as an alternative treatment.

Histological examination of the liver showed nodular cirrhosis, focal bridging fibrosis, steatosis, canalicular and hepatocellular cholestasis, siderosis, tubular transformation

of hepatocytes, hemosiderin deposits, giant cell hepatitis with multinucleated giant cells, and even hepatocellular carcinoma in these patients. Electron microscopy demonstrated accumulation of mitochondria with reduction of cristae<sup>1-6,10-15</sup>. In our patient, postmortem liver tissue revealed severe hepatocellular damage, cholestasis and fibrosis (Fig. 1).

In conclusion, we report here a novel mutation of the *DGUOK* gene in the family of a Turkish patient with mtDNA depletion syndrome. This mutation was associated with poor prognosis, early clinical symptoms and death. mtDNA depletion syndrome should always be considered in a newborn and/or infant patient with treatment-resistant liver failure.

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