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Case Report

Hepatocerebral Mitochondrial DNA Depletion Syndrome: Clinical and Morphologic Features of a Nuclear Gene Mutation

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Maternally inherited mitochondrial DNA (mtDNA) requires deoxyribonucleotides (dNTP) within the organelle to replicate. mtDNA codes for the organelle's ribosomal proteins, tRNAs, and 13 of its respiratory chain proteins (1). Nuclear DNA codes for the remainder of the mitochondrial oxidative phosphorylation enzymes and for the two deoxyribonucleoside kinases needed to salvage nucleotides and maintain the organelle's dNTP pools. Therefore, mutations in either the nuclear or mitochondrial genomes can impair mitochondrial function and cause a wide spectrum of clinical phenotypes (1,2).

In 1991, Moraes et al. (3) first described mitochondrial DNA depletion syndrome (MDS), a condition defined by defective oxidative phosphorylation associated with low levels of mtDNA. Subsequently, a number of investigators have described the clinical, biochemical, and morphologic features of MDS in liver (4–8). Infants present with poor oral intake and failure to thrive. In addition to the standard biochemical indicators of liver dysfunction, severe lactic acidosis and ketotic hypoglycemia occur. Some infants experience hypotonia, neuromuscular weakness, and nystagmus. Hepatic pathology in children with MDS includes microvesicular steatosis, canalicular cholestasis with bile duct thrombi, ductular proliferation, glycogen depletion, and occasionally hepatocellular cholestasis. An oncocytic appearance seen in certain hepatocytes results from the accumulation of abnormal mitochondria (8). Ultrastructural findings have been described in detail (9) and include hepatocytes containing an increased number of swollen mitochondria with abnormal cristae. However, an indistinguishable clinical, biochemical, and morphologic form of liver failure has also been described in a family with a mitochondrial respiratory chain defect and normal levels of mtDNA (10).

The MDS phenotype can be expressed in a single organ, such as liver or muscle, or be more generalized (1,2). How a single molecular defect can lead to multiple syndromes with phenotypic expression in different organs has yet to be elucidated. There are two mitochondrial kinases that are required for mtDNA replication and maintenance of mtDNA levels (11,12). Both are under the control of nuclear genes. A myopathic form of MDS has been linked to the gene for thymidine kinase-2 (*TK2*) (11). Some patients with hepatocerebral MDS have mutations in the deoxyguanosine kinase gene (*dGK*) that yields liver failure with (12,13) or without central nervous system involvement (13). However, *dGK* mutations were not found in livers from three families (12), and in only three of 21 livers from infants with hepatocerebral MDS (13).

The current report describes the clinical features and histologic and ultrastructural characteristics in a family with hepatocerebral MDS secondary to a *dGK* mutation. The correlation between the type of mutation in *dGK* and the phenotypic presentation of MDS is also discussed.

CASE REPORTS

Three related Palestinian infants presented with similar clinical features of weakness, poor feeding, and poor weight gain. Figure 1 shows the pedigree of this family. Liver abnormalities began in the first weeks of life.

Patient 1

The first affected child in this family was a 2.44-kg girl born full term by normal spontaneous vaginal delivery without complications. An older sibling was healthy.

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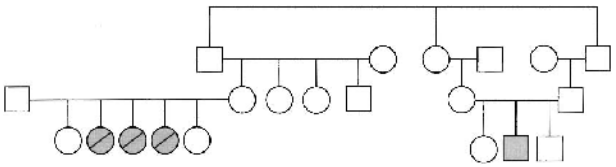

FIG. 1. Pedigree of hepatocerebral MDS. Circles and squares denote females and males, respectively. Shaded symbols denote affected individuals. A diagonal line denotes that individual is deceased. The oldest two affected sisters are Patients 1 and 2. The male cousin is Patient 3.

Patient 1 was hospitalized twice during the first 2 months of life for dehydration, diarrhea, and acidosis without hypoglycemia. Sepsis workup and stool cultures were negative. Several formula changes were made before referral to Children's Medical Center of Brooklyn when the patient was 2½ months of age.

On initial examination the infant was thin, irritable, and anicteric. She was in no acute distress and had no dysmorphic features. Her weight was 2.64 kg, an increase of only 200 g since birth. She had reached appropriate milestones but appeared thin and wasted in the extremities. Results of abdominal examination were unremarkable, with no hepatic or splenic enlargement. She had axial hypotonia, normal deep tendon reflexes bilaterally, and bilateral rotatory nystagmus with random eye movements. Treatment for gastroesophageal reflux and high calorie oral feeds were started. Two weeks later, she continued to have poor oral intake and experienced hypoglycemia (serum glucose, 30–50 mg/dL), which necessitated admission for hydration and initiation of nasogastric feedings. At that time her weight was 3 kg. Liver function tests included SGOT (250 IU/L), SGPT (159 IU/L), total bilirubin (2.9 mg/dL), total protein (5.2 g/dL), and albumin (3.3 g/dL). There were no episodes of apnea, bradycardia, or seizures during the hospitalization.

The results of diagnostic tests were as follows: alpha-1-antitrypsin, 253 mg/dL (normal, 140–430 mg/dL); alpha-fetoprotein, <2 ng/mL; serum pyruvate, 0.2 mg/dL (normal, 0.3–0.7 mg/dL); total serum carnitine, 42 μmol/L (normal, 24–100 μmol/L); and free serum carnitine, 33 μmol/L (normal, 20–88 μmol/L). Plasma and urine amino acid profiles were normal. Slight elevation in long chain fatty acids was noted: C24/C22, 1.31 (normal, 0.6–1) and C26/C22, 0.038 (normal, 0.001–0.02). Urine organic acid analysis showed multiple nonspecific abnormalities, including increased levels of several C6 to C10 dicarboxylic acids, thought to be secondary to medium chain triglyceride supplementation. Abdominal ultrasound showed no abnormalities of the liver or kidneys. Ophthalmoscopy revealed sharp disks, borderline pale retina, no cataracts, and no cherry red spot. She had a normal electroencephalogram and head magnetic resonance imaging scan. Visual evoked potentials were absent on one side, and brain stem evoked potentials were normal bilaterally.

Light microscopic evaluation of a liver biopsy performed at almost 6 weeks of life, revealed a variation in the intensity of the usual eosinophilic staining of hepatocyte cytoplasm. There was microvesiculation and swelling of an estimated 60% of hepatocytes and possibly of some Kupffer cells. The affected cells were periodic acid-Schiff negative. These changes were particularly prominent in the periportal and midlobular zones. In addition, there was pseudoacinar transformation; focal ground glass appearance and dropout of hepatocytes with focal destruction of the limiting plate; bi- and trinucleated hepatocytes; hepatocanalicular cholestasis; periportal fibrosis with bridging and extension into the lobule; hemosiderin deposition; and mild macrosteatosis. In view of the absence of inflammation and the negative orcein stain, these features were felt to indicate an inborn error of metabolism (Fig. 2A, B) Electron microscopic analysis demonstrated focal dilatation of mitochondrial cristae (Fig. 3). After discharge, there was progressive worsening of her liver function test results, with transaminases reaching the levels of more than 400 units/mL and profound coagulopathy unresponsive to vitamin K.

At 5 months, she presented to the emergency department with hematemesis, profound hypoglycemia, apnea, and shock. Her mother had discontinued nasogastric feedings 2 hours earlier. She was resuscitated, intubated, started on broad spectrum antibiotics, and given multiple transfusions. She was noted to have coagulopathy as part of her liver dysfunction and experienced seizures on her fifth hospital day. On her eighth hospital day, she experienced severe hematochezia and could not be resuscitated.

Patient 2

The next sibling of Patient 1 was ultimately subjected to biochemical and molecular analyses and is the proband of this family. She was born at term, weighing 2,040 g, and had Apgar scores of 8 and 9. She experienced hypoglycemia, acidosis, and poor feeding and was transferred to the neonatal intensive care unit on day 1 of life. Laboratory test on day 2 were aspartate transaminase (SGOT), 163 IU/L; alanine transaminase (SGPT), 83 IU/L; ammonia, 66 (g/dL; total bilirubin, 12.8 mg/dL; and direct bilirubin, 1.9 mg/dL. Titers for toxoplasmosis, rubella, cytomegalovirus and herpes were negative (TORCH). She was given continuous nasogastric tube feeding at night and frequent boluses during the day. She adapted well to the feeding regimen with normal glucose levels and appropriate weight gain. Plasmalogens and very long chain fatty acids, which had not been studied in the sibling, were normal. The light microscopic and ultrastructural features of liver biopsy specimen at 3 weeks of life were similar to but more marked than those of her deceased sibling at 3 months.

At 4 months of age, she was admitted for possible sepsis, failure to thrive, and hypotonia. She was too weak

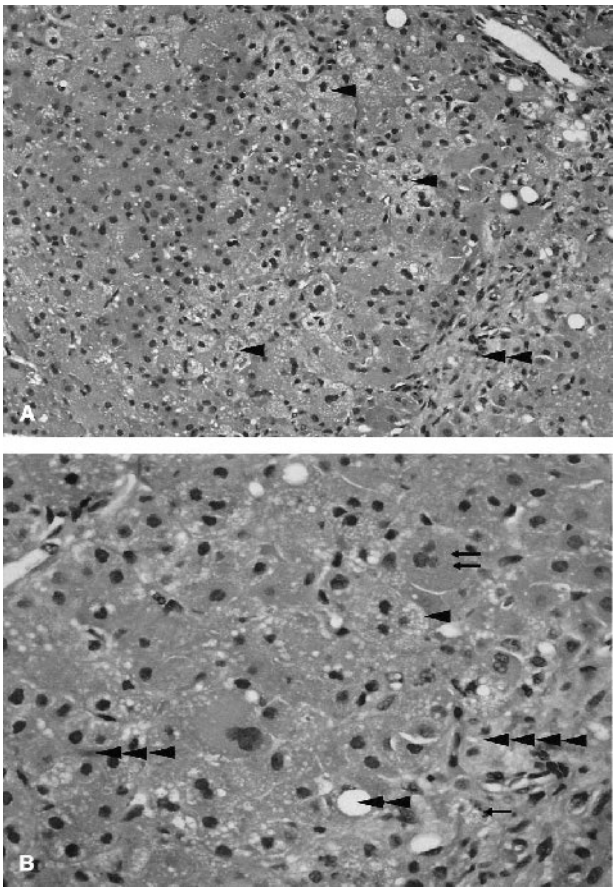

FIG. 2. (**A**) Hematoxylin and eosin-stained light micrograph of the liver (original magnification, ×200). There is variable intensity in the cytoplasmic eosinophilic staining and microvesicular change in focal clusters of hepatocytes (arrowhead). These are adjacent to relatively normal-appearing hepatocytes. There is focal fibrosis (double arrowhead). (**B**) Hematoxylin and eosin-stained light micrograph of the liver (original magnification, ×400). The prominent features include microvesicular change (arrowhead); macrovesicular steatosis (double arrowhead); intracanicular bile plug (triple arrowhead); inflammatory cell aggregate (beneath quadruple arrowhead); hemosiderin within Kupffer cells (arrow); trinucleated hepatocyte (double arrows).

to roll over and could barely hold up her head. All cultures were negative. Total carnitine was normal (34 (mol/L) with a borderline low level (20 (mol/L) of free carnitine. At 6 months, she was hospitalized with hematemesis and coagulopathy. She was found to have hypoglycemia, acidosis, anasarca, and end stage liver disease. No varices were noted on endoscopic analysis. Sepsis workup yielded negative results, and she died on the 12th hospital day after experiencing an upper gastrointestinal bleeding episode.

Enzyme studies from a muscle biopsy specimen obtained at the time of death revealed a decrease in cytochrome *c* oxidase, succinate cytochrome *c* reductase, NADH-cytochrome *c* reductase, NADH-dehydrogenase and succinate dehydrogenase activity. Citrate synthase activity was normal. Southern blot analyses of liver tissue showed severe (90%) mtDNA depletion with a mtDNA to nuclear DNA ratio of 0.1. Sequencing of the *dGK* gene region yielded a homozygous GATT duplication of nucleotides 766 to 768, resulting in a truncated 255 amino acid enzyme. For additional details see "Patient 1" in Mandel et al. (12). A third child in this family was conceived and died in early infancy in Palestine with a similar clinical course.

Patient 3

Patient 3 was a first cousin of Patients 1 and 2, had consanguineous parents (first cousins). He had presented before Patient 2 was diagnosed to Children's Memorial Hospital in Chicago. He was delivered at term after an uncomplicated pregnancy. He weighed 2.7 kg. At 10 days of age, he presented to an emergency room with direct hyperbilirubinemia and was subsequently admitted. Testing revealed a negative sweat test result, normal alpha-1-antitrypsin levels, normal newborn screen for galactosemia, negative urinary succinylacetone, normal karyotype, and negative serologies for hepatitis A, B, and C, Epstein-Barr virus, and human immunodeficiency virus. TORCH titers were normal, and a urine culture was negative. An abdominal ultrasound was normal. The patient had no excretion on HIDA scan. Pertinent laboratory findings included: hemoglobin of 8.1 g/dL, total bilirubin of 14 mg/dL with direct bilirubin of 8 mg/dL, an elevated gamma-glutamyl-transpeptidase of 127 IU/L, prothrombin time (PT) of 14.5 seconds, partial thromboplastin time of 52.6 seconds, fibrinogen of 46 (mol/L, SGPT of 72 IU/dL, and an elevated alpha-fetoprotein

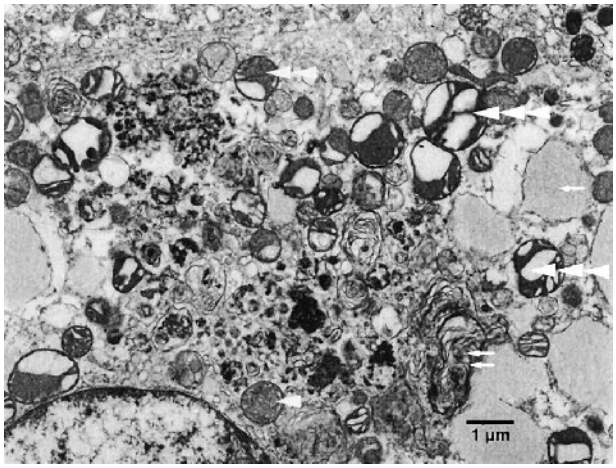

FIG. 3. Uranyl acetate and lead citrate stained electron micrograph of a hepatocyte (original magnification, ×4,292). The prominent features include relatively normal mitochondrion (arrowhead); mitochondrion with dilated cristae (double arrowhead); mitochondria with severely dilated cristae and dense matrix (triple arrowheads); fat vesicle (arrow); and myeloid figure (double arrows).

level of 85,000 ng/mL. At 3 weeks of age, a liver biopsy revealed individual cell necrosis and steatosis. There was evidence of iron overload, but a buccal biopsy did not demonstrate excessive salivary gland iron, thus excluding neonatal hemochromatosis as a primary diagnosis. Electron microscopic analysis of the liver biopsy specimen was interpreted as showing normal mitochondria and mild steatosis. The patient was readmitted at 2 months of age for failure to thrive. His laboratory test results were similar, except that his albumin was 1.8 g/dL.

At 3 months, the liver was enlarged, ammonia levels had risen to 200 (g/dL, and the PT had increased to 17 seconds. The tip of the spleen was palpable, but there was no ascites. The patient was floppy with a head lag but could interact with the examiner and move all four extremities. A repeat liver biopsy at that time revealed acinar transformation, microvesicular fatty change, intrahepatic cholestasis, and mild to moderate periportal fibrosis. His condition continued to deteriorate, and he experienced acute respiratory distress syndrome. He received an orthotopic liver transplant and spent several months in the pediatric intensive care unit on a ventilator. Studies performed after liver transplantation showed significant deterioration of visual and auditory pathways. Despite tube feeding and supplementation with carnitine and fat-soluble vitamins, he experienced a steady deterioration. After a minor pulmonary bleeding episode, he died of multiorgan failure. Liver rejection was ruled out by several biopsies, yet severe vascular congestion was noted.

DISCUSSION

A previous report retrospectively examined 22 patients with inborn errors of hepatic oxidative phosphorylation, i.e., mitochondrial diseases (14). The authors divided the patients into those with early onset who had severe neurologic involvement and fatal outcome, and those with delayed onset who had variable, and minor if any neurologic involvement and a more favorable outcome. Six cases of children receiving liver transplants for mitochondrial disease without extrahepatic manifestations have been reported (15). Five children did well, including all of those who survived the perioperative period (15). Most authorities suggest that patients with hepatic mitochondrial disease and neurologic symptoms should not be offered liver transplantation (14–16).

Hepatocerebral MDS is a subgroup of inborn errors of hepatic oxidative phosphorylation that also contains two distinct phenotypes. Individuals with both phenotypes have had mutations mapped to the *dGK* gene (13). Some *dGK* mutations lead to a premature termination of translation, resulting in a protein without any enzyme activity (12,13). This genotype results in mtDNA depletion in liver and other tissues (12,13). One of the patients in the current report had neurologic symptoms in addition to liver failure. He received a liver transplant but died with progressive central nervous system deterioration. Several cousins had similar clinical presentations, and one was found to have a termination type of *dGK* mutation (13). Another identified *dGK* mutation is a point mutation in the enzyme's active site, resulting in a complete protein with compromised catalytic activity. This type of *dGK* mutation leads to a milder phenotype that is restricted to the liver. A child with this mutation received a successful orthotopic liver transplant at 17 months (13).

Even among this small cohort, who presumably all had the same type of mutation, there is some variability in both biochemical parameters (elevation in alpha-fetoprotein in Patient 3 but not in Patient 1) and ultrastructural findings (normal mitochondria described in early biopsy of Patient 3 with abnormal organelles noted in Patient 1 and reported in most published reports). Whether the disease progression is influenced by other genetic factors, environmental, or nutritional factors will be determined when larger numbers of affected families are described.

Only a small proportion of hepatocerebral MDS appears to be related to the *dGK* locus (12,13). Future work should identify additional genes involved in this phenotype and may also correlate clinical severity with type of mutation. Thus, the clinician may be guided in the management of a greater proportion of children affected with hepatocerebral MDS. A recent publication has postulated that autosomal recessive Navajo neurohepatopathy is another example of the MDS phenotype with several forms (17). Future investigations should determine whether the phenotypic variation in this disease also corresponds to different types of mutations in the same genetic locus.

A single report documented prenatal findings that were later confirmed MDS (18). Prenatal diagnosis was based on immunocytochemical staining of an mtDNA-encoded protein from cultured amniocytes. However, MDS was established by the measurement of mtDNA in liver and muscle after birth. As measuring mtDNA levels from amniocytes or from chorionic villus sampling is currently not commercially available, this procedure is not an option for prenatal diagnosis in families at risk for MDS. The identification of defined genetic mutations leading to the MDS phenotype will now permit accurate prenatal testing in families with the affected genotypes.

Recently, the mother of patients 1 and 2 became pregnant again. Nuclear DNA isolated from amniotic fluid was subjected to dGK gene sequencing and revealed no mutations. This finding was confirmed postnatally on the unaffected infant's blood.

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REFERENCES

1. Sokol RJ, Treem WR. Mitochondria and childhood liver diseases. *J Pediatr Gastroenterol Nutr* 1999;28:4–16.
2. Hirano M, Vu TH. Defects of intergenomic communication: where do we stand? *Brain Pathol* 2000;10:451–61.
3. Moraes CT, Shanske S, Tritkschler H-J, et al. mtDNA depletion with variable tissue expression: a novel genetic abnormality in mitochondrial diseases. *Am J Hum Genet* 1991;48:492–501.
4. Mazziotta MRM, Ricci E, Bertini E, et al. Fatal infantile liver failure associated with motochondrial DNA depletion. *J Pediatr* 1992;121:896–901.
5. Maaswinkel-Mooij PD, Van den Bogert C, Scholte HR, et al. Depletion of mitochondrial DNA in the liver of a patient with lactic acidemia and hypoketotic hypoglycemia. *J Pediatr* 1996;128: 679–83.
6. Bakker HD, Scholte HR, Dingemans KP, et al. Depletion of mitochondrial deoxyribonucleic acid in a family with fatal neonatal liver disease. *J Pediatr* 1996;128:683–7.
7. Morris AMA, Taanman JW, Blake J, et al. Liver failure associated with motochondrial DNA depletion. *J Hepatol* 1998;28:556–63.
8. Ducluzeau PH, Lachaux A, Bouvier R, et al. Depletion of mitochondrial DNA associated with infantile cholestasis and progressive liver fibrosis. *J Hepatol* 1999;30:149–55.
9. Mandel H, Hartman C, Berkowitzet D, et al. The hepatic mitochondrial DNA depletion syndrome: ultrastructural changes in liver biopsies. *Hepatology* 2001;34:776–84.
10. Goncalves I, Hermans D, Chretien D, et al. Mitochondrial respiratory chain defect: a new etiology for neonatal cholestasis and early liver insufficiency. *J Hepatol* 1995;23:290–4.
11. Saada A, Shaag A, Mandel H, et al. Mutant mitochondrial thymidine kinase in mitochondrial DNA depletion myopathy. *Nature Genet* 2001;29:342–44.
12. Mandel H, Szargel R, Labay V, et al. The deoxyguanosine kinase gene is mutated in individuals with depleted hepatocerebral mitochondrial DNA. *Nature Genet* 2001;29:337–41.
13. Salviati L, Sacconi S, Mancuso M, et al. Mitochondrial DNA depletion and dGK gene mutations. *Ann Neurol* 2002;52:311–7.
14. Cormier-Daire V, Chretien D, Rustin P, et al. Neonatal and delayed-onset liver involvement in disorders of oxidative phosphorylation. *J Pediatr* 1997;130:817–22.
15. Dubern B, Broue P, Dubuisson C, et al. Orthotopic liver transplantation for mitochondrial respiratory chain disorders: a study of five children. *Transplantation* 2001;71:633–7.
16. Thompson M, Mckiernan P, Buckels J, et al. Generalised mitochondrial cytopathy is an absolute contraindication to orthotopic liver transplant in childhood. *J Pediatr Gastroenterol Nutr* 1988; 26:478.
17. Vu TH, Tanji K, Holve SA, et al. Navajo neurohepatopathy: a mitochondrial DNA depletion syndrome? *Hepatology* 2001;34: 116–20.
18. Blake JC, Taanman JW, Morris AMM, et al. Mitochondrial DNA depletion syndrome is expressed in amniotic fluid cell cultures. *Am J Pathol* 1999;155:67–70.