Hepatocerebral Form of Mitochondrial DNA Depletion Syndrome

Novel MPV17 Mutations

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Background: Autosomal recessive mutations in MPV17 (OMIM *137960) have been identified in the hepatocerebral form of mitochondrial DNA depletion syndrome (MDS).

Objective: To describe the clinical, morphologic, and genetic findings in 3 children with MPV17-related MDS from 2 unrelated families.

Results: Two children were homozygous for nonsense mutation p.W120X. A third child was compound heterozygous for missense mutation p.G24W and for a macrodeletion spanning MPV17 exon 8. All patients demonstrated lactic acidosis, hypoglycemia, hepatomegaly, and progressive liver failure. Neurologic symptoms manifested at a later stage of the disease. Death occurred within the first year of life in all 3 patients.

Conclusions: These data confirm that MPV17 mutations are associated with a 2-stage syndrome. The first symptoms are metabolic and rapidly progress to hepatic failure. This stage is followed by neurologic involvement affecting the central and peripheral systems.

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Mitochondrial DNA depletion syndrome (MDS) is characterized by a quantitative abnormality of the mitochondrial genome inherited as an autosomal recessive trait. Although decreased in amount, residual mitochondrial DNA (mtDNA) does not harbor any mutations or rearrangements. Because the primary abnormality resides in a nuclear gene but the target DNA is mitochondrial, MDS is an example of a group of mitochondrial disorders labeled as defects of intergenomic communication. Some individuals with MDS have muscle weakness, others demonstrate liver failure, and still others manifest multisystem involvement and lactic acidosis. Consistent with different phenotypes, mtDNA depletion can affect specific tissues (most commonly muscle or liver) or multiple organs, including the heart, brain, and kidneys. Since 2001, MDS has been linked to mutations in 8 nuclear genes. Mutations in TK21 (OMIM *188250) and RRM2B2 (OMIM *604712) are associated with early-onset myopathy with or without renal proximal tubulopathy. Mutations in SUCLA13 (OMIM *611224) and SUCLA24 (OMIM *603921), encoding isofoms of succinyl–coenzyme A lyase (a Krebs-cycle enzyme), have been associated with encephalomyopathy, while mutations in Twinkle (OMIM *60675), POLG (OMIM *174763), DGUOK (OMIM *601465), and MPV175-10 cause combined encephalopathy and liver failure. A variant form of hepatoneural MDS affects the Navajo population (Navajo neurohepatopathy) and has been associated with a mutation in MPV17.11,12 Herein, we describe the clinical, molecular morphological, and biochemical features of 3 children (from 2 unrelated families) with hepatocerebral MDS secondary to novel MPV17 mutations.

METHODS

Routine muscle and liver biopsy specimen histologic examinations were performed as previously described.11,12 Respiratory chain activities were also measured as previously described.
described. Genomic DNA from patients’ tissues was extracted using standard protocols. Real-time quantitative polymerase chain reaction (PCR) was used to evaluate mtDNA content in liver and muscle biopsy specimens. For MPV17 sequence analysis, 7 coding exons and the exon-intron boundaries of the gene were amplified and sequenced directly as previously described. Primers used for the long PCR that expands the region of exon 7 and downstream of exon 8 were 5'-GCCAACCTTACCTGGTCCCCCTT-3' and 5'-GATCACGAGGTCAGTAGTTTGAA-3'. The sequence variants found in the patients were evaluated in available relatives, as well as in 100 consecutive control subjects (200 alleles).

REPORT OF CASES

CASE 1

The third child of consanguineous Iraqi parents was born at 41 weeks after an uncomplicated pregnancy. Her birth weight was 3770 g, and the Apgar scores were 9, 10, and 10. Family history was positive for 2 miscarriages. The infant was hospitalized twice during the first 2 months of life for dehydration and diarrhea without hypoglycemia. At 2 months, she developed jaundice, and her liver was enlarged to 4 cm below the right costal margin. She was small for her age (weight and length were in the 10th percentiles) and microcephalic. She had a broad nasal bridge but no other dysmorphic features. Relevant laboratory findings included the following: signs of coagulopathy (quick time, 40%), increased serum concentration of \( \alpha \)-fetoprotein (360 000 ng/mL) (to convert \( \alpha \)-fetoprotein concentration to micrograms per liter, multiply by 1.0), markedly impaired absorption of fat-soluble vitamins (vitamin A level, 8 µg/dL) (to convert vitamin A level to micromoles per liter, multiply by 0.0349), hyperbilirubinemia (total bilirubin level, 8.4 mg/dL; and direct bilirubin level, 5.8 mg/dL) (to convert bilirubin levels to micromoles per liter, multiply by 17.104), and high levels of aspartate aminotransferase (203 U/L; reference range, 12-27 U/L) and alanine aminotransferase (91 U/L; reference range, 7-28 U/L) (to convert aspartate aminotransferase and alanine aminotransferase levels to microkatal per liter, multiply by 0.0167). Brain magnetic resonance (MR) imaging at 3 months of age was normal. An initial liver biopsy was performed at the same age.

When she was readmitted at the age of 4 months, failure to thrive was evident, and her liver function had worsened. She developed hypoglycemia (glucose level, 22 mg/dL) (to convert glucose level to millimoles per liter, multiply by 0.0555) and lactic acidosis, with a lactate concentration of up to 54 mg/dL in plasma (reference range, 6.3-18.9 mg/dL) and up to 72 mg/dL in cerebrospinal fluid (reference range, <19.82 mg/dL) (to convert lactate concentration to millimoles per liter, multiply by 0.111). A second liver biopsy specimen at age 6 months showed progression of hepatic degeneration, and brain MR imaging revealed cytotoxic edema involving the subcortical white matter of both frontal lobes, more severe on the right (Figure 1). Magnetic resonance spectroscopy showed a slight but significantly elevated lactate peak within the parietooccipital white matter (Figure 2). Because of her severe and rapidly progressive liver disease and mild neurologic involvement, patient 1 was considered a candidate for liver transplantation. However, while on the transplant list, she developed severe metabolic crisis with hyperammonemia, lactic acidosis, and massive coagulation disorder. She also developed nephrolithiasis, and her neurologic status...
worsened, with the development of dystonic movement. She died at age 11 months of liver failure.

**CASE 2**

This patient, the younger sister of patient 1, was born after an uneventful pregnancy and birth (weight, 3970 g; length, 56 cm; head circumference, 36 cm; and Apgar scores of 9, 10, and 10). Prenatal diagnosis had been offered to the parents but was refused. This child first showed clinical signs at 7 weeks of age, when she was first admitted because of severe failure to thrive, and at age 3 months she had severe dystrophy (3870 g). Her liver was palpable 4 cm below the costal margin. Apart from severe muscular hypotonia, no specific neurologic symptoms were found. Laboratory investigations revealed severe lactic acidosis (\( \geq 144 \) mg/dL in plasma) and cholestasis (total bilirubin level, 0.53 mg/dL; direct bilirubin level, 0.44 mg/dL; total bile acid level, 132 mg/dL [reference range, 0-4.92 mg/dL]; lipoprotein X level, 98 mg/dL [reference range, 0-11 mg/dL]) [to convert bile acid level to micromoles per liter, multiply by 2.448]; and lipoprotein X level, 98 mg/dL [reference range, 0-11 mg/dL] [to convert lipoprotein X level to micromoles per liter, multiply by 0.0337]), and her liver function was deteriorating (quick time, 23%; international normalized ratio, 2.7; aspartate aminotransferase level, 266 U/L [reference range, 10-35 U/L]; alanine aminotransferase level, 148 U/L [reference range, 10-35 U/L]; and glutamate dehydrogenase level, 110 U/L [reference range, 0-7 U/L]). Patient 2 died of liver failure at 5 months.

**CASE 3**

This patient was born at full term (a 2.7-kg girl) by normal spontaneous vaginal delivery. She was the only child of unrelated parents, and conception was by artificial insemination with the father’s sperm because of a history of endometriosis in the mother. The infant cried immediately after birth, but she was noted to feed poorly and to be shaky and jittery. She was discharged home at 2 days of life, but she was readmitted a few days later because of lethargy. Laboratory findings at that time revealed low glucose level, high plasma tyrosine and plasma lactate levels, and abnormal liver functions with coagulopathy. Brain MR imaging at 2 weeks showed bilateral subdural hemorrhages with an area of periventricular leukomalacia involving the right parietal region. Liver and skeletal muscle biopsy specimens were obtained. Because of low blood glucose levels, she underwent a fasting study at 2 months of age, which was stopped after 5.5 hours. Her lowest documented glucose level was 38 mg/dL. She was maintained on nasogastric feedings of an elemental formula every 3 hours, avoiding periods of overnight fasting. On physical examination at age 5 months, she appeared small for her age. She was hypotonic, with roving eye movements and nystagmus. She had severe failure to thrive and hepatomegaly (the liver edge was 5.5 cm below the right costal margin). A formal ophthalmologic examination showed nystagmus but normal fundus and optic discs. Her echocardiogram, electrocardiogram, renal function, and brainstem auditory-
and visual-evoked responses were normal. Her condition worsened, and she died of liver failure at 9 months of age.

RESULTS

In patient 1, histologic examination of an initial liver biopsy specimen obtained at 3 months of age revealed cholestasis, cell necrosis, mild microvesicular steatosis, and peripoortal fibrosis with inflammation (Figure 2A and C). These findings were much more marked at 6 months of age in a second biopsy specimen and had evolved to micronodular cirrhosis (Figure 2B and D). In patient 3, a liver biopsy specimen showed fibrosis, steatosis, cholestasis, hemosiderosis, glycogen storage, and hepatocellular swelling. Findings in a muscle biopsy specimen were 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.

In patients 1 and 3, real-time PCR of liver tissue showed severe reduction in the ratios of mtDNA to nuclear DNA. There was 70% depletion in patient 1 and 84% depletion in patient 3.

Molecular genetic analysis showed 3 novel mutations in MPV17. Patients 1 and 2 had a homozygous G to A transition at nucleotide position 359 of exon 5. Their parents were heterozygous. The mutation changes the p.W120 into a TAG stop codon and is predicted to result in a truncated protein. Patient 3 was compound heterozygous. One allele (from the father) carried a missense mutation that changes a highly conserved G to T (p.G24W). The allele from the mother carried a 1.5-kb deletion that spanned from intron 7 well into exon 8 (Figure 3). All 3 mutations were absent in 100 healthy control subjects.

COMMENT

Mitochondrial DNA deletion syndrome differs from other mitochondrial disorders because it is a quantitative defect rather than a qualitative deficit. The low copy number of mtDNA in some tissues is likely to cause insufficient synthesis of respiratory chain components. Primary MDS is transmitted as an autosomal recessive trait and has been associated with mutations in 8 nuclear genes. Five of them (TK2, RRM2B, SUCLA1, SUCLA2, and DGUOK) encode nuclear products needed for DNA replication, and POLG and twinkle products function directly at the mtDNA replication fork. MPV17 encodes an inner membrane mitochondrial protein of unknown function.

The clinical spectrum of MDS is diverse. In some patients, only 1 organ is affected, while in others the syndrome is multisystemic. The liver seems particularly vulnerable to MPV17 mutations, as all described patients herein shared severe hepatopathy as a common clinical feature. As in our 3 cases, patients manifest hepatomegaly and progressive liver failure within the first weeks of life, leading to death a few months later. Initial symptoms include vomiting, diarrhea, failure to thrive, or developmental delay. Coagulopathy, hypoglycemia, lactic acidosis, elevated total and conjugated bilirubin levels, and high levels of serum alanine aminotransferase and aspartate aminotransferase are common. However, other organs are not spared, especially the brain. Most patients who initially showed isolated hepatopathy without clinical or neuroradiological signs of central nervous system involvement subsequently developed neurologic symptoms at a later stage of the disease; these included ataxia, tremor, dystonia, neuropsychiatric, and altered ocular movement such as multidirectional nystagmus. In patients 1 and 2 described herein, neurologic involvement also manifested in a second stage of the disease. Patient 1 developed dystonic movements associated with alterations seen on MR imaging and MR spectroscopy at age 7 months. In patient 2, the only neurologic sign at the age of 3 months was hypotonia, which may be of central origin but could be the result of severe muscle wasting. Patient 2 died of liver failure at 5 months. Patient 3 developed microcephaly, hypotonia, and nystagmus. Severe hepatic involvement in association with MDS has been described in patients carrying DGUOK or POLG1 mutations. Patients with POLG mutations typically have neurologic symptoms at the time of their first clinical ex-
amination. In patients with features of Alpers-Huttenlocher syndrome on MR imaging, the most prominent symptom is intractable progressive epilepsy, while hepatopathy develops later in the course of the disease but may worsen rapidly, especially after exposure to valproic acid. However, we have also detected POLG mutations in children who were primarily admitted because of fulminant liver problems and minor neurologic problems in whom deterioration of cerebral functions occurred after liver transplantation.\textsuperscript{16,17} In DGUOK deficiency, liver involvement seems to be the most prominent feature, leading to liver cirrhosis and causing early-onset liver failure.

MPV17 mutations associated with hepatic MDS were first described in 2006.\textsuperscript{9} Since then, 15 cases from 11 families have been ascribed to 7 different pathogenic MPV17 mutations.\textsuperscript{9-11} Three were frameshift mutations, and 4 were missense mutations. We identified 3 additional MPV17 mutations. One was a nonsense mutation (the first described thus far to our knowledge) that resulted in a truncated protein abolishing the last 56 amino acids. The second was a missense mutation affecting a highly conserved amino acid in the first transmembrane domain of the protein. The third was a macrodeletion covering part of the seventh intron and the eighth exon of the gene. These data show that MPV17-related MDS is genetically heterogeneous, although the C\textsubscript{148} to G\textsubscript{149} dinucleotide of the MPV17 sequence has been postulated to be a mutational hot spot.\textsuperscript{12}

The treatment of acute liver failure and progressive liver disease in mitochondrial hepatopathies remains unsatisfactory. Present treatments involve the use of various vitamins, cofactors, and respiratory substrates, none of which have proven effective. Supportive treatments may include infusion of sodium bicarbonate for acute metabolic acidosis, intravenous administration of glucose, or nasogastric feedings to avoid fasting hypoglycemia.\textsuperscript{18,19} Cornstarch administration can also protect against fasting hypoglycemia (Rossella Parini, MD; oral communication; May 2006). Liver transplantation is a treatment option for end-stage liver disease associated with MDS, but the role of liver transplantation in mitochondrial hepatopathy is controversial, largely because of the multisystemic nature of this disorder. The few available studies show mixed outcomes, with a survival rate of less than 50%.\textsuperscript{20-23} The presence of extrahepatic involvement is a subsequent independent event. Therefore, the absence of extrahepatic features of mitochondrial disease at the time of liver transplantation does not warrant a good outcome even if the transplant is successful. On the other hand, a few patients with isolated liver involvement have undergone successful liver transplantation.\textsuperscript{23} Therefore, careful screening of potential organ recipients is crucial because systemic involvement portends poor long-term prognosis. Recently, MR imaging and MR spectroscopy have proven to be valuable tools in the evaluation of central nervous system involvement in candidates for transplant because of acute liver failure due to mitochondrial hepatopathy.\textsuperscript{26} Our study reinforces the concept that MPV17 mutations are associated with rapidly progressive infantile hepatic failure and that neurologic involvement is a subsequent independent event.

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Author Contributions: Dr Zeviani had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Spinazzola, Santer, Schaef er, Ding, and Zeviani. Acquisition of data: Spinazzola, Santer, Akman, Tsiakas, Ding, Karadimas, Shanske, Ganesh, and Di Mauro. Analysis and interpretation of data: Spinazzola, Santer, Akman, Schaef er, Di Mauro, and Zeviani. Drafting of the manuscript: Spinazzola, Shanske, and Ganesh. Critical revision of the manuscript for important intellectual content: Santer, Akman, Tsiakas, Schaef er, Karadimas, Di Mauro, and Zeviani. Obtained funding: Ding and Zeviani. Administrative, technical, and material support: Santer, Akman, Tsiakas, Schaef er, and Karadimas. Study supervision: Spinazzola, Shanske, Di Mauro, and Zeviani.

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**Announcement**

**Trial Registration Required.** In concert with the International Committee of Medical Journal Editors (ICMJE), *Archives of Neurology* will require, as a condition of consideration for publication, registration of all trials in a public trials registry (such as http://ClinicalTrials.gov). Trials must be registered at or before the onset of patient enrollment. This policy applies to any clinical trial starting enrollment after July 1, 2005. For trials that began enrollment before this date, registration will be required by September 13, 2005, before considering the trial for publication. The trial registration number should be supplied at the time of submission.

For details about this new policy, and for information on how the ICMJE defines a clinical trial, see the editorial by DeAngelis et al in the January issue of *Archives of Dermatology* (2005;141:76-77). Also see the Instructions to Authors on our Web site: www.archneurol.com.