Navajo Neurohepatopathy Is Caused by a Mutation in the MPV17 Gene

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Navajo neurohepatopathy (NNH) is an autosomal recessive disease that is prevalent among Navajo children in the southwestern United States. The major clinical features are hepatopathy, peripheral neuropathy, corneal anesthesia and scarring, acral mutilation, cerebral leukoencephalopathy, failure to thrive, and recurrent metabolic acidosis with intercurrent infections. Infantile, childhood, and classic forms of NNH have been described. Mitochondrial DNA (mtDNA) depletion was detected in the livers of two patients, suggesting a primary defect in mtDNA maintenance. Homozygosity mapping of two families with NNH suggested linkage to chromosome 2p24. This locus includes the MPV17 gene, which, when mutated, causes a hepatocerebral form of mtDNA depletion. Sequencing of the MPV17 gene in six patients with NNH from five families revealed the homozygous R50Q mutation described elsewhere. Identification of a single missense mutation in patients with NNH confirms that the disease is probably due to a founder effect and extends the phenotypic spectrum associated with MPV17 mutations.
weakness, stocking-glove sensory loss, and areflexia; nerve conduction studies confirmed the presence of a peripheral neuropathy. Muscle biopsy revealed neurogenic atrophy. He did not have corneal ulcerations or scarring. He had no evidence of hepatomegaly or liver dysfunction, but his serum γ-glutamyltransferase (GGT) was mildly elevated. By age 12 years, he had lost the ability to walk and to swallow, which required him to be placed in a chronic care facility, where he was fed through a gastrostomy tube. He developed hepatic cirrhosis and died of liver failure at age 16 years.

As an infant girl, patient 2 (V3) (reported as patient 12 by Holve et al.1) had recurrent hypoglycemia and metabolic acidosis with febrile illnesses. At age 6 mo, it was noted that she had poor growth and delayed motor development. At age 2 years, when she first walked, she had distal limb muscle weakness, wasting, and areflexia. Nerve conduction studies revealed diffusely slow velocities. Serum transaminases and GGT were two to three times the upper limits of normal, and total and direct bilirubin were mildly elevated. Her weakness worsened and, by age 4 years, she could move only by pulling herself across the ground. Because of distal sensory loss, she had acral mutilation complicated by recurrent skin infections. She also had corneal anesthesia and developed corneal ulcers. Her IQ was 72. Brain magnetic resonance imaging (MRI) re-

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**Figure 1.** Families 1–5 with NNH. Family 1 was described by Singleton et al.4 and by Holve et al.1 The probands in families 4 (II-1) and 5 (II-1) were reported as patients 1 and 2, respectively, by Vu et al.3 The nucleotide 149G→A transition in the MPV17 gene is denoted by a plus sign (+), and the normal sequence is denoted by a minus sign (−).
revealed diffuse leukoencephalopathy, which was particularly prominent in the cerebellum. By age 16 years, she needed a wheelchair because of her neuropathy and had poor vision due to corneal scarring. At age 20 years, she had hematemesis, and evaluation revealed esophageal varices and cirrhosis. She died of liver failure.

Patient 3 (II-6 in family 2) was reported as patient 17 by Holve et al. This boy had classic NNH. He had an older sister (reported as patient 1 by Holve et al.) who died of liver failure at age 1 year and who was presumed to have NNH. In infancy, he had gross motor delay and poor growth; he began walking at age 2 years, and his height and weight were <5th percentile. At age 3 years, he developed corneal ulcerations, and medical evaluation revealed signs of peripheral neuropathy, including loss of corneal sensation, stocking-glove sensory loss, distal limb weakness, and areflexia. Nerve conduction studies revealed marked slowing, and a sural nerve biopsy showed severe loss of myelinated fibers. Blood tests revealed elevated transaminases and GGT but normal bilirubin. At age 4 years, he contracted varicella, which was complicated by liver failure, acute respiratory distress syndrome, metabolic acidosis, and renal dysfunction, but he responded to intensive care. Subsequently, he had persistently elevated transaminases—two to three times the upper limit of normal—and mild elevations of total and direct bilirubin, and his neuropathy progressively worsened. By age 10 years, he was unable to walk or to feed or dress himself. Acral mutilation due to sensory neuropathy caused recurrent infections. He was fed through a gastrostomy tube. Brain MRI revealed leukoencephalopathy. At age 14 years, he had hematemesis due to esophageal varices and cirrhosis. One year later, he died of hepatic failure.

Patient 4 (individual II-1 in family 3) was reported as patient 4 by Holve et al. This girl had infantile NNH and a second cousin (reported as patient 2 by Holve et al.) who died of liver failure at age 1 year and who was presumed to have NNH. In our study, all patients met described diagnostic criteria for NNH. A genomewide scan was performed using a set of 400 polymorphic DNA microsatellite markers at average distances of 10 cM (ABI Prism Linkage Mapping Set MD-10 [Applied Biosystems]). The PCR products were run on an ABI Prism 310 Genetic analyzer. Genotypes were iden-
genes involved in mitochondrial functions identified involved in the maintenance of mtDNA copy number. drial endonuclease G (ENDOG) [MIM 600879]) and mitochondrial endonuclease G (ENDOG [MIM 600440])—that are involved in the maintenance of mtDNA copy number.

We then considered other candidate genes around chromosomal locus D2S305, a region that contains several genes involved in mitochondrial functions identified through integrative genomics. One of them was MPV17 (MIM 137960), which has been recently associated with a new hepatocerebral form of MDS. The close proximity of this gene to our chromosomal locus—and the striking similarities between the phenotype of the described patients with MPV17 mutations and the clinical picture of infantile NNH—prompted us to perform direct mutation analysis of this gene in our patients. Sequencing revealed a homozygous R50Q mutation in our patients 1–6. Further genetic analysis of all available unaffected individuals confirmed segregation of the mutation with the disease. Taken together, the results of linkage analysis and the detection of the same pathogenic R50Q mutation developed multiple brain lesions—prompted us to perform direct mutation analysis of this gene to our chromosomal locus—and the striking similarity to the patients with classic NNH. Because of the inevitable progression of neurological dysfunctions, liver transplantation has very limited value in treatment of this disease. Neuropathy, a salient diagnostic feature of NNH, is not common in other forms of infantile MDS. Only patients with the benign, later-onset myopathic form of MDS due to TK2 mutation and patients with Alpers syndrome (MIM 203700) and POLG mutations show subclinical neuropathy.

The phenotype of the six patients with MDS described by Spinazzola et al. resembles one end of the NNH clinical spectrum that is dominated by lethal early-onset hepatopathy. Two of four affected infants in the Italian family who had the same homozygous R50Q mutation as our patients with NNH died before age 9 mo. Similar courses were seen in patients 2-4 and 3-1 in the same report. Notably, the two surviving patients harboring a homozygous R50Q mutation developed multiple brain lesions and had delayed growth, both typical symptoms of NNH. On the other hand, patient 4-1 developed progressive spinocerebellar ataxia, myoclonus of hands and feet, mental retardation, and severe neurogenic kyphoscoliosis, which are not among the clinical features of NNH. Interestingly, the prolonged survival of this patient to age 9 years, which was achieved by dietary control for hypoglycemia, may have therapeutic implications for patients with the infantile and childhood forms of NNH.

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* Two-point LOD >~1.0 at θ = 0 for all 10 markers.
This phenotypic variability suggests that additional neurohepatocerebral forms of MDS may be caused by mutations in MPV17 in non-Navajo patients. Alternatively, the prominent peripheral nervous system involvement in NNH may indicate that additional unlinked modifier genes or epigenetic factors play a role in the determination of the phenotype.

Liver samples from two patients with NNH have been assessed for mtDNA depletion and respiratory-chain defects. Both showed marked depletion (89% and 82% reductions relative to controls) and corresponding reduced activities of respiratory-chain complexes with mtDNA-encoded subunits. Because liver from one patient with infantile NNH and from another with the classic form have been the only ones studied, it is not possible to correlate the severity of the mitochondrial defects with the NNH subtypes. It is noteworthy that the mtDNA depletion in liver of patients with NNH is similar to that observed in liver of patients with Alpers syndrome (60%–97% mtDNA depletion) due to POLG mutations.15–17 with hepatocerebral disease due to DGUOK mutations (61%–98% mtDNA depletion),4,18 and with hepatic disease due to MPV17 mutations (>70% mtDNA depletion).10

In conclusion, we identified, by homozygosity mapping, the genetic defect causing NNH, showing that a single mutation in exon 2 of MPV17 accounts for the different forms of the disease. The identification of MPV17 as the disease-causing gene will provide a definitive pre- and postnatal diagnosis of NNH and will shed light on the pathogenic mechanism of the disorder.

Acknowledgments

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Web Resource

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for NNH, DGUOK, TK2, SUCLA2, POLG, TFAM, NRF-1, ENDOG, MPV17, and Alpers syndrome)

References