Liver disease in Navajo neuropathy

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Objective: To describe clinical and histologic features of liver disease in infants and children with Navajo neuropathy (NN).

Methods: Physicians at Navajo Area Indian Health Service facilities and neurologists and gastroenterologists at regional referral hospitals were surveyed for identification of patients born between 1980 and 1994 with known or suspected NN. Clinical records and liver histologic findings were reviewed.

Results: Liver disease was present in all children with NN. Three clinical phenotypes of NN were observed, based on age at presentation and course: *infantile NN* presented in 5 infants before 6 months of age with jaundice and failure to thrive and progressed to liver failure before 2 years of age; *childhood NN* presented in 6 children between 1 and 5 years of age with liver dysfunction, which progressed to liver failure and death within 6 months; and *classical NN* presented in 9 children with variable onset of liver disease but progressive neurologic deterioration. Liver histologic findings were characterized by multinucleate giant cells, macrovesicular and microvesicular steatosis, pseudo-acini, inflammation, cholestasis, and bridging fibrosis and cirrhosis. Cases of all 3 phenotypes occurred within the same kindred.

Conclusions: Liver disease is an important component of NN and may be the predominant feature in infants and young children. We propose changing the name of this disease to *Navajo neurohepatopathy.* (J Pediatr 1999;135:482-93)

Navajo neuropathy was first described in 1976 by Appenzeller at al,[1] and a more extensive clinical description was provided by Singleton et al[2] in 1990. Salient features of the disorder included a progressive sensorimotor neuropathy in full-blooded Navajo children, manifested by distal weakness, areflexia, and a loss of sensation that resulted in corneal ulcerations and acral mutilation. In addition, many patients had intermittent elevation of liver enzymes or Reye's syndrome–like episodes. Despite extensive investigation, the etiology of NN remains unknown. Family studies suggest that NN is an inherited metabolic disease with autosomal recessive inheritance. This disorder appears to occur only in Navajo Indians of the southwestern United States.

A1AT	α_1-Antitrypsin
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
FTT	Failure to thrive
GGT	γ-Glutamyltransferase
MRI	Magnetic resonance imaging
NN	Navajo neuropathy
PFIC	Progressive familial intrahepatic cholestasis
PT	Prothrombin time

Recent advances in molecular genetics have demonstrated that inherited disorders of a specific genotype can have variable phenotypic expression. In the past 15 years, we have seen a number of Navajo infants who presented with idiopathic liver disease who later developed the sensorimotor neuropathy and corneal ulcerations typical of NN. In addition, we have identified a group of Navajo children, most of whom were relatives of patients with known NN, with many of the neurologic findings characteristic of NN who died of liver failure in infancy or childhood. A sural nerve biopsy specimen from one of these younger patients (Fig 1) demonstrated the loss of large- and small-caliber myelinated fibers characteristic of NN.[1,2] We hypothesized that these infants and young children with fatal liver disease, as well as the older patients with predominantly neurologic findings as previously described,[1,2] all have phenotypic variants of the same genetic

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disease confined to Navajos. The purpose of this report is to characterize the previously underappreciated severe liver involvement and its effect on survival in infants, as well as older children, with NN.

CLINICAL PHENOTYPES

Twenty patients have been diagnosed with NN on the Navajo Indian Reservation from 1980 to 1994. The subjects of this report include 9 children with typical NN as described by Appenzeller et al[1] and Singleton et al[2] and 11 children with NN who died of liver failure in infancy and childhood (Table I).

Although there is an overlap of clinical findings, we have divided patients with NN into 3 phenotypic groups based on age at onset of symptoms, the primary clinical presentation, and the course of the disease: *classical NN*, the group originally described by Appenzeller et al,[1] in which liver disease may be noted in infancy but progressive neurologic deterioration is the major clinical feature; *infantile NN*, in which jaundice and failure to thrive in infancy are followed by progression to liver failure and death within the first 2 years of life; and *childhood NN*, in which the acute onset of severe hepatic dysfunction between the ages of 1 and 5 years leads to liver failure and death within months. The following case summaries illustrate the typical course of each presentation.

Infantile NN: Patient 4

Patient 4 was a Navajo infant born at term to a woman, gravida 1, para 1, by spontaneous vaginal delivery without difficulty. Family history was notable for a second cousin with NN. At 4 months of age, weight gain was poor; at 6 months of age, weight and height were below the 5th percentiles, and jaundice was noted. Physical examination showed mild hepatomegaly and some peripheral muscle wasting. No neurologic deficits were noted. Laboratory investigation revealed aspartate aminotransferase of 367 IU/L, alanine aminotransferase of 136 IU/L, alkaline phosphatase of 424 IU/L, cholesterol of 227 mg/dL, total protein/albumin of 6.7/3.4 g/dL, γ-glutamyltransferase of 101 IU/L, bilirubin (total/direct) of 7.4/4.7 mg/dL, and a prothrombin time of 21 seconds. Arterial blood gas demonstrated a metabolic acidosis. A liver biopsy specimen showed significant cholestasis and fibrosis. Evaluation for causes of liver failure showed normal urine and serum amino and organic acid levels, normal copper metabolism, and normal carnitine levels; α_1-antitrypsin phenotype was MM. Urine grew cytomegalovirus, and serum cytomegalovirus IgG was positive; other viral serologies were negative. Over the next month, the child had worsening jaundice with hepatic decompensation at 7 months of age, culminating in a successful liver transplantation. Posttransplant liver function was excellent; however, FTT and metabolic acidosis persisted. Evaluation at 16 months of age showed developmental delay, hypotonia, and decreased deep-tendon reflexes. The diagnosis of NN was entertained but initially dismissed when physical examination revealed no corneal scarring or sensory neuropathy, cranial magnetic resonance imaging showed no demyelination, and nerve conduction velocities were found to be normal. At 3 years of age, the child still had marked FTT, metabolic acidosis, and gross motor delay—she could stand with assistance but could not walk independently. Physical examination showed corneal anesthesia. Motor neuropathy was manifested by peripheral muscle atrophy, hypotonia, weakness, and absent deep-tendon reflexes and was confirmed by marked delay in nerve conduction velocity. Sensory neuropathy was evident with decreased response to deep and sharp pain. Magnetic resonance imaging of the brain was normal.

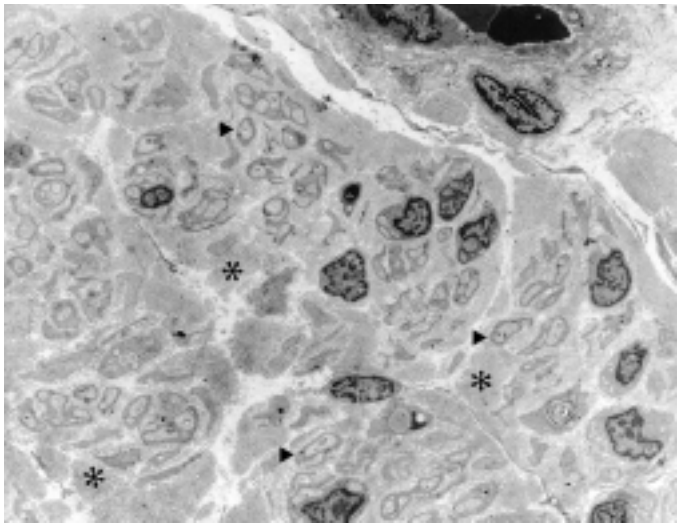

Fig 1. Ultrastructural features of severe sural nerve lesion in NN. Electron microscopic image of sural nerve biopsy specimen from patient 11 at 30 months of age. Note severe reduction of large- and small-caliber myelinated nerve fibers, with increased collagen *(asterisks)* dispersed among small unmyelinated axons *(arrowheads)* (original magnification ×5100).

Childhood NN: Patient 6

The patient was a 3760-g infant born at term to a woman, gravida 4, para 3, without complications. Family history was notable for a sibling with NN. She was well until 3 months of age when she was noted to be below the 5th percentile for weight and height. Results of physical and laboratory evaluation for FTT were unremarkable. Gross motor delay became evident because

Table I. Clinical features of NN

Patient No.	Age at onset (mo)	Presenting features	Nerve conduction velocity slowing	Cranial imaging demyelination	Clinical course	Affected relatives
Infantile NN						
1	2	Jaundice, FTT	ND	ND	Recurrent metabolic acidosis; died of liver failure at age 1 y.	Sibling
2	1	Jaundice, FTT	–	–/+	At age 10 mo developed liver failure; liver transplant with death from acute rejection of liver.	Second cousin
3	1	Jaundice, FTT	+	+	Died of liver failure at age 11 mo.	Second cousin
4	6	Jaundice, FTT	+	–	At age 9 mo developed liver failure; successful liver transplant with progression of neurologic disease.	Second cousin
5	4	Jaundice, FTT	+	–/+	Recurrent metabolic acidosis; died of liver failure at age 20 mo.	Second cousin
Childhood NN						
6	36	Jaundice, ascites	+	–	Presented in liver failure with cirrhosis and died in 3 mo.	Sibling
7	36	Jaundice	+	+	Presented in liver failure and cirrhosis and died in 4 mo.	Sibling
8	12	Jaundice	ND	+	Presented in liver failure and cirrhosis and died in 2 mo.	Second cousin
9	24	Jaundice	ND	+	Presented in liver failure and sepsis and died in 2 mo.	None
10	26	Ascites	+	+	Presented in liver failure and died in 2 mo.	None
11	16	Corneal ulcers	+	ND	FTT and areflexia; sudden onset of liver failure at age 4 y, progressing to death in 3 mo.	None
Classical NN						
12	6	FTT	+	++	FTT, developmental delay, and sensorimotor neuropathy. No clinical liver disease at age 16 y.	Second cousin
13	4	Hypoglycemic seizure	+	+	Infantile cirrhosis; developed hepatocellular carcinoma at age 11 y and underwent liver transplantation; neurologic symptoms developed after transplantation and then resolved; now age 16 y.	Sibling
14	4	RS-like episode	+	–	After RS-like episode, developed cirrhosis; died of respiratory arrest at age 12 y.	Sibling
15	1	Jaundice, FTT	+	–	Neonatal hepatitis with no subsequent clinical liver disease, but developed progressive neurologic disease at age 14 y.	Sibling
16	5	RS-like episode	+	-	Progressive neurologic disease; died of cirrhosis at age 7 y.	Sibling

Continued on page 485.

Table I. Continued

Patient No.	Age at onset (mo)	Presenting features	Nerve conduction velocity slowing	Cranial imaging demyelination	Clinical course	Affected relatives
Classical NN						
17	36	Corneal ulcers, FTT	+	++	Hepatic failure with varicella infection at age 3 y; no further clinical liver disease, but progressive neurologic disease at age 11 y.	Sibling
18	16	Corneal ulcers	ND	–	No clinical liver disease, but progressive neurologic disease at age 11 y.	Sibling
19	7	RS-like episode, FTT	–	ND	No clinical liver disease, but progressive neurologic disease at age 9 y.	Sibling
20	10	Corneal ulcers	+	++	Cirrhosis on liver biopsy at age 2 y; elevated AFP at age 6 y, but no hepatic cancer; progressive neurologic disease present at age 8 y.	None

ND, Not done; *AFP,* α-fetoprotein; -, abnormality absent; *RS,* Reye's syndrome; +, abnormality present; -/+, initial brain MRI normal and second MRI abnormal, as described by Williams et al.[4]

she did not walk until 2 years of age; fine motor skills, speech, and personal-social development were normal. By 3 years of age, she had lost the ability to walk independently and had developed contractures in both hands. She presented at this time with the acute onset of ascites and mild jaundice. Laboratory values showed an AST of 115 IU/L, ALT of 35 IU/L, alkaline phosphatase of 418 IU/L, GGT of 70 IU/L, bilirubin (total /direct) of 3.7/2.6 mg/dL, and a PT of 22 seconds. Physical examination showed ascites without palpable liver or spleen. There was loss of peripheral muscle mass, peripheral weakness, hypotonia, contractures of both hands, and absent deep-tendon reflexes. No corneal scarring was appreciated. Serology for hepatitis A and B viruses, cytomegalovirus, and Epstein-Barr virus were normal. A1AT phenotype was MM; results of copper studies and the serum vitamin E level were normal. A liver biopsy specimen showed the presence of giant cells, lobular disarray, and cirrhosis. Nerve conduction velocity showed marked slowing with decreased amplitude. A sural nerve biopsy specimen showed loss of large- and small-caliber myelinated fibers. A computerized tomographic scan of the brain showed no obvious demyelination. Esophageal varices were found during endoscopy. The family declined the option for liver transplantation, and the patient died of liver failure 3 weeks later. Autopsy request was denied.

Classical NN: Patient 20

The patient was a term infant born to a woman, gravida 6, para 5. No other siblings or relatives were noted to have hepatic or neurologic disease. At 10 months of age, the child presented with decreased corneal sensation and bilateral corneal ulcerations. There was no known antecedent trauma. Bacterial and viral cultures of the cornea were negative. Frequent application of Lacrilube (Allergan) to the eye and the performance of bilateral tarsorrhaphies led to healing of the corneal ulcerations. Mild hypotonia and developmental delay were noted, but peripheral sensation and deep-tendon reflexes were normal. Despite the absence of clinical signs or symptoms of hepatic disease, AST was 112 IU/L, ALT 69 IU/L, GGT 427 IU/L, alkaline phosphatase 243 IU/L, and bilirubin (total/direct) 1.0/0.2 mg/dL with normal albumin and PT values. Cranial magnetic resonance imaging showed increased T_2-weighted signal in the periventricular area, consistent with demyelination. A tentative diagnosis of NN was made. At 2 years of age, he was noted to have a wide-based gait, peripheral weakness, decreased peripheral sensation, and areflexia. Nerve conduction velocities showed slowing, and a sural nerve biopsy specimen showed marked loss of myelinated fibers. Liver was enlarged and firm on examination, and aminotransferases were twice the upper limit of normal. A liver biopsy specimen showed portal tract inflammation and cirrhosis. Over the next 3 years, the child demonstrat-

Table II. Diagnostic criteria for NN

1. *Sensory neuropathy* demonstrated by physical examination, acral mutilation, or sural nerve biopsy
2. *Motor neuropathy* demonstrated by physical examination, denervation on EMG, or delayed motor nerve conduction velocity
3. *Corneal anesthesia* by ophthalmologic examination, corneal ulcers, or scarring
4. *Liver disease:* seronegative hepatitis, Reye's syndrome–like episodes, liver failure, or abnormal liver biopsy findings
5. Documented metabolic or infectious derangement including FTT, short stature, delayed puberty, or systemic infection (sepsis, meningitis, pyelonephritis, or disseminated viral infection)
6. Evidence of central nervous system demyelination on radiologic imaging

Definite case: (a) Four of six above criteria present or (b) three of above criteria and a sibling with NN

Modified from Singleton et al. Neurology 1990;40:368.

Table III. Specialized tests evaluated in study subjects

Test	No. of patients tested
Urine and serum organic acid and amino acid concentrations	12*
Serum acylcarnitine profile	3
α_1-Antitrypsin phenotype	6
Leukocyte lysosomal enzymes	5
Serum transferrin isoelectric focusing (carbohydrate-deficient glycoprotein syndrome)	5
Serum very long chain fatty acid concentrations	4
Fibroblast plasmalogen synthesis	4
Respiratory chain enzyme activity in skin fibroblasts	3
Liver copper concentrations and serum ceruloplasmin levels	3
Serum carnitine and free carnitine concentrations	3

Results of all tests were normal or negative.
*Includes 3 patients with infantile NN and severe liver disease who had mildly elevated dicarboxylic acid concentrations in urine, consistent with concentrations expected in severe liver disease.

ed poor growth with height and weight below the 5th percentile. He also developed decreased peripheral sensation with acral mutilation, foot drop, and increasing difficulty walking. By 5 years of age, braces and a walker were required for ambulation. At 6 years of age, the liver was palpable 2 cm below the right costal margin and hard. Total protein, albumin, and PT were normal; and AST was 60 IU/L, ALT 49 IU/L, GGT 400 IU/L, and alkaline phosphatase 165 IU/L. As part of a screening program for hepatocellular carcinoma in patients with NN, an α-fetoprotein level was found to be markedly elevated at 1315 ng/mL. Computerized tomography of the liver showed multiple hypodense nodules in the liver, raising the question of cirrhosis with regenerating nodules versus multi-focal carcinoma. A biopsy specimen of the liver lesion, obtained with a computerized tomography–guided needle, showed cirrhosis. In the 2 years of subsequent follow-up, the child has remained stable with no clinical worsening of hepatic function, no complications of cirrhosis, and no evidence of hepatic carcinoma.

METHODS

Study Design

The Navajo Area Indian Health Service provides medical care and maintains health records for nearly all Native Americans residing on the Navajo Reservation. Physicians at all Navajo Area Indian Health Service facilities, as well as pediatric neurologists and gastroenterologists at tertiary referral hospitals in Phoenix and Albuquerque, were surveyed for patients born between 1980 and 1994 who had known or probable cases of NN, as well as any Navajo children who died of unexplained liver failure. Data were obtained by a retrospective review of medical records by using a standard checklist for signs and symptoms at presentation, clinical course, and laboratory evaluations.

Criteria for diagnosis (Table II) were modified from the description by Singleton et al.[2] Corneal anesthesia as demonstrated by ophthalmologic examination, even without evidence of corneal scarring or ulceration, was added as a positive eye finding. Recent experience has shown that decreased corneal sensation is the precursor to corneal scarring in NN, just as decreased peripheral sensation leads to acral mutilation. Second, evidence of central nervous system demyelination by imaging was added as a diagnostic criterion. Cranial imaging shows that central nervous system demyelination is a frequent finding in NN.[3,4] Traditional Navajo beliefs have precluded autopsy examination of affected brain tissue.

To be included as a case patient, a subject was required to be full-blooded Navajo, have the onset of signs or symptoms of disease before age 10 years, and have no other known cause of liver disease. Furthermore, each patient had to satisfy either 4 of the 6 criteria for case definition (Table II) or 3 of the criteria and have a sibling with NN. We identified 21 patients in 14 families. Complete records were available for 20 of the patients who constitute the subjects of this

Table IV. Liver test results (mean ± SEM) at clinical presentation of NN

	AST (IU/L)	ALT (IU/L)	Alkaline phosphatase (IU/L)	GGT (IU/L)	Bilirubin total/direct (mg/dL)	PT (s)
Infantile NN	250 ± 41	142 ± 15	554 ± 64	113 ± 18	7.8 ± 1.9	15 ± 1.6
Childhood NN	321 ± 193	162 ± 121	582 ± 143	88 ± 16	6.4 ± 2.2	26 ± 4.1*
Classical NN	126 ± 15*	70 ± 10*	425 ± 132	242 ± 47*	1.9 ± 0.6*	12.7 ± 0.1
Normal range	12-48	4-35	100-320	5-35	<1.0/<0.4	12-14

*P < .05 versus the other 2 groups calculated by Kruskal-Wallis test for non-parametric data.

report. The incidence of NN was calculated by using birth statistics for the years 1980 to 1994 for the western Navajo Nation from the Navajo Area Indian Health Service Office of Planning and Evaluation.

Liver Histologic Findings

Liver histologic findings from biopsy, autopsy, or hepatectomy specimens were reviewed by 2 of the investigators (R.W.T. and R.J.S.). Tissue was available for review from 13 of the study subjects and was scored, with investigators blinded to the clinical and laboratory information, by a scoring system developed for this disease. Liver sections were scored in a semi-quantitative manner from 0 (not present) to 4 (markedly abnormal) by criteria set up for each of 11 histologic features, which included hepatocellular necrosis, ballooning degeneration, hepatocellular cholestasis, canalicular cholestasis, presence of multi-nucleated giant cells, macrovesicular steatosis, microvesicular steatosis, fibrosis, inflammation, pseudo-acinar formation, and bile duct proliferation. (Details of the scoring system are available from the authors by request.) Electron micrographs of liver specimens, available from 4 subjects, were also reviewed.

Statistical Analysis

Liver histology scores and liver blood test values were compared among the study groups by the Kruskall-Wallis test for non-parametric data. Correlations between histologic variables were sought by linear regression analysis with the least-squares technique. A P value of .05 was considered statistically significant. Data were expressed as the mean ± SEM.

Table V. Histologic scores for liver specimens from patients with NN

	Clinical phenotypic group		
Histologic feature	Infantile (n = 4)	Childhood (n = 3)	Classic (n = 6)
Hepatocyte necrosis	1.4 ± 0.6	1.7 ± 0.3	0.8 ± 0.3
Hepatocyte ballooning	2.1 ± 0.5	2.7 ± 0.3	1.6 ± 0.3
Hepatocellular cholestasis	0.8 ± 0.3	1.5 ± 0.3	1.0 ± 0.5
Canalicular cholestasis	1.4 ± 0.8	1.0 ± 0.6	0.7 ± 0.5
Multinucleate giant cells	1.3 ± 0.6	2.3 ± 1.2	0.5 ± 0.3
Macrovesicular steatosis	1.3 ± 0.6	1.2 ± 0.8	0.9 ± 0.4
Microvesicular steatosis	1.5 ± 0.5	2.0 ± 1.0	1.3 ± 0.3
Fibrosis	3.0 ± 0.4	4.0 ± 0	2.6 ± 0.5
Inflammation	2.1 ± 0.4	2.3 ± 0.3	1.5 ± 0.2
Pseudo-acinar formation	1.4 ± 0.5	1.0 ± 0	0.3 ± 0.2*
Bile duct proliferation	0.2 ± 0.2	2.7 ± 1.3*	0.7 ± 0.5

All values are expressed as mean ± SEM.
*P < .05 versus the other 2 groups calculated by the Kruskal-Wallis test for non-parametric data. Each histologic feature scored from 0 (absent) to 4 (severely abnormal) by criteria defined for each feature. Complete scoring system available from the authors by request.

RESULTS

Results of evaluation for rare disorders causing both liver and neurologic involvement were negative (Table III). Clinical, laboratory, and histologic features of patients with infantile, childhood, and classical NN are listed in Tables I, IV, and V.

Infantile NN

Patients with infantile NN presented between the ages of 1 and 6 months with FTT and jaundice. At presentation, all 5 patients in this group showed hepatomegaly without splenic enlargement. Serologic testing excluded known viral causes. Modest elevations of AST, ALT, and GGT levels were present at presentation. Synthetic liver function, as measured by PT and serum albumin levels, was normal in 3 patients and mildly impaired in 2. Serum cholesterol and triglyceride levels were normal. Serum lactate levels were elevated during intercurrent illnesses in the 3 patients tested and slightly elevated when patients were well.

Liver biopsy findings available from 4 patients were characterized by prominent pseudo-acinar formation of hepatocytes, canalicular cholestasis, and

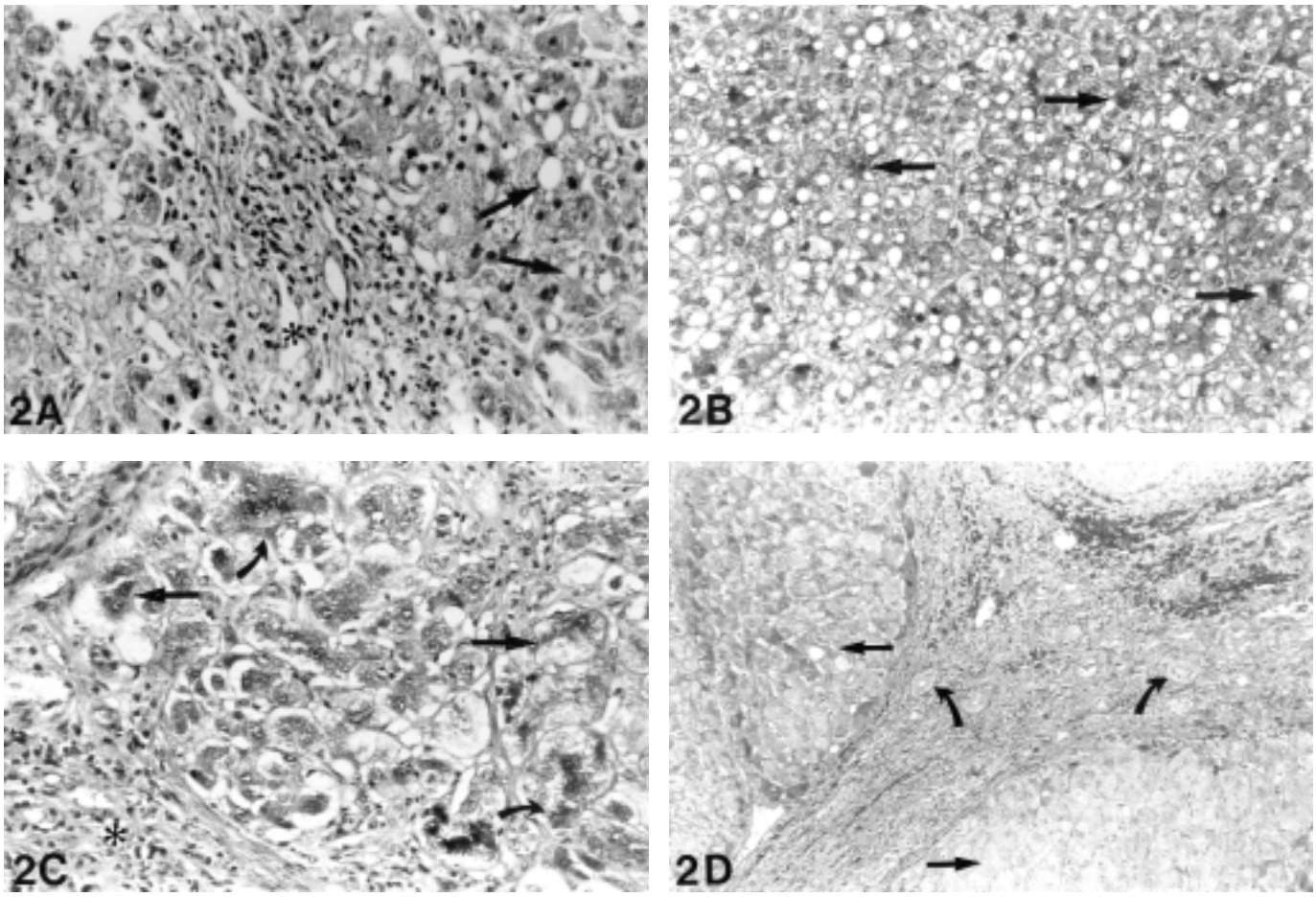

Fig 2. Histologic features of liver lesion in NN. **A,** Patient 2 with infantile form of NN at 9 months of age. There is prominent portal fibrosis and mononuclear cell portal tract inflammation *(asterisk)*. Note mixture of microvesicular and macrovesicular steatosis in hepatocytes *(arrows)* (hematoxylin and eosin, original magnification ×200). **B,** Patient 10 with childhood form of NN at 26 months of age. Note striking microvesicular and some macrovesicular steatosis. Degenerating hepatocytes are prominent *(arrows)* (trichrome, original magnification ×200). **C,** Patient 8 with childhood form of NN at 14 months of age. High-power view of multinucleate giant cells *(arrows)*, many with dark granular intracellular bile *(curved arrows)*. Note portal tract expansion by fibrous connective tissue and inflammation *(asterisk)* (trichrome, original magnification ×200). **D,** Patient 13 with classic form of NN at 24 months of age. Regenerative nodules *(arrows)* are separated by fibrous connective tissue with proliferating bile ducts *(curved arrows)*. A hepatocellular carcinoma was present in a nearby area of the liver (not shown) (trichrome, original magnification ×100).

macrovesicular steatosis (Fig 2, *A*). Ballooning degeneration of hepatocytes, multinucleate giant cells, microvesicular steatosis, portal tract and lobular inflammation (lymphocytes and neutrophils), and bridging fibrosis were prominent. Evaluation of bile ducts showed no abnormal proliferation, and possible paucity of interlobular bile ducts was observed in specimens from 2 patients. All 5 patients had progression to liver failure by 10 months of age.

Clinical neuropathy was absent at presentation but became evident over time. By 9 months of age, all of the patients demonstrated weakness and gross motor delay. All 5 patients developed areflexia, a classic finding in NN. Vitamin E deficiency as a possible cause of these neurologic symptoms was ruled out by normal serum α-tocopherol and lipid concentrations in 3 of the 4 patients tested. Corneal scarring was not present; however, 2 patients demonstrated corneal anesthesia on examination.

Imaging and electrophysiologic studies confirmed the neurologic progression in this group of patients. Cranial MRI scans from patients 2 and 5 were normal before 5 months of age; however, repeat scans at 9 months of age showed increased signal on T_2-weighted images in the cerebellar and subcortical white matter. This signal pattern was believed to represent demyelination and was consistent with the imaging findings described for NN.[3,4] A similar pattern of central nervous system demyelination was seen on an MRI scan from patient 3 at 9 months of age. Peripheral neuropathy was demonstrated with slowing of nerve conduction velocity in 3 of 3 patients tested after 9 months of age. Patient 2 had normal findings on a sural nerve biopsy specimen at age 10 months. Patient 3 underwent post-mortem examination of ulnar nerve at 11 months of age, which showed loss of myelinated fibers similar to that described in patients with NN.[1]

The most striking example of pro-

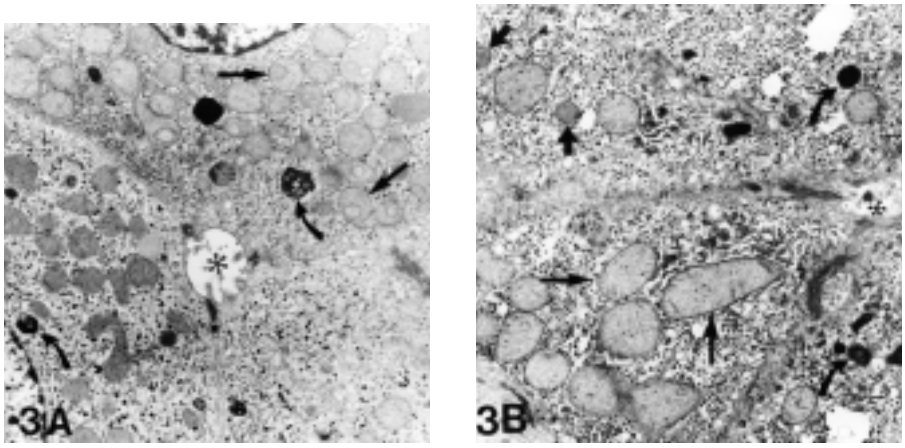

Fig 3. Ultrastructural features of liver lesion in NN. **A,** Patient 16 at 6 months of age. Ringed mitochondrial cristae are present *(arrows)*. Bile pigment is noted in hepatocyte cytoplasm *(curved arrows)*. Central bile canaliculus shows decreased microvilli *(asterisk)* (original magnification ×17,000). **B,** Patient 14 with classic NN at 47 months of age. Ultrastructure shows loss of mitochondrial cristae *(arrows)*. Peroxisomes are present *(short arrows)*. Bile pigment is present in hepatocyte cytoplasm *(curved arrows)* (original magnification ×17,000).

gression of neurologic abnormalities in a case of infantile NN was patient 4. No neurologic findings were noted at liver transplantation at age 9 months. At 16 months of age, she demonstrated peripheral weakness and areflexia. By 3 years of age, all of the neuropathic findings of NN were present: decreased peripheral sensation with skin injury, corneal anesthesia, and progressive motor neuropathy demonstrated by loss of peripheral strength and muscle mass, confirmed by marked slowing of nerve conduction velocity.

Childhood NN

Patients with childhood NN presented during early childhood with acute, aggressive liver disease, which progressed within months to hepatic failure and death. Five of the 6 patients had only modest elevation of AST, ALT, and GGT values, despite evidence of significant impairment of synthetic function as demonstrated by decreased serum albumin levels and prolonged PT values. All patients appeared to have had a mild viral illness just before sudden hepatic decompensation. Serologic studies for hepatitis A, B, and C viruses, cytomegalovirus, Epstein-Barr virus, and adenovirus were negative. Liver histology obtained from 2 patients showed the most prominent histologic abnormalities in 8 of 11 categories evaluated, compared with the other 2 forms of NN (Table IV). These included hepatocyte necrosis, hepatocyte ballooning, hepatocytic cholestasis, multinucleate giant cells, microvesicular steatosis, portal tract inflammation, bile duct proliferation, and fibrosis (Fig 2, *B* and *C*). Specimens from one child (patient 10) showed significant bile duct proliferation associated with cirrhosis.

In only one patient (No. 11) was the diagnosis of NN established before the onset of liver disease, based on the presence of FTT, corneal ulcerations, and sensorimotor neuropathy. This patient had no known liver disease until ascites developed, which progressed to liver failure and death within 3 months. The other 5 patients did not have specific neurologic deficits identified before the onset of liver disease, although all clearly had gross motor delay (inability to walk independently until 18 months of age). At the time of evaluation of liver disease, signs of neuropathy were clearly evident. Examination by pediatric neurologists documented motor neuropathy (peripheral weakness and areflexia) in all 5 patients, slowing of nerve conduction velocities in the 3 patients tested, and peripheral sensory neuropathy in all 5 patients. Nerve biopsy specimens showed generalized loss of myelinated fibers in 2 of 3 patients tested. Central nervous system demyelination was detected by computerized tomography or MRI in 4 of 5 patients. Nutritional status in all patients was normal, as assessed by examination, weight-for-height percentiles, and serum retinol and α-tocopherol levels.

Classical NN

The courses of the 9 patients with classical NN were similar to those described in earlier reports,[1-3] with striking neuropathic findings, motor neuropathy, and slowed nerve conduction velocity. Review of the records of these patients showed that liver disease was more prevalent than previously described. All patients except one had clinical liver disease identified at some time in the course of their illness, and all had chronic elevation of aminotransferases, but with somewhat higher (4-10 times normal) GGT levels than observed in patients with infantile and childhood NN. Serologies for hepatitis B, hepatitis C, and Epstein-Barr viruses were negative in all 9 patients. In addition, 5 of the 9 patients (Nos. 13, 14, 15, 16, and 19) had evidence of liver disease in infancy, which preceded neuropathic findings. Diagnosis of NN was not established until 4 years of age, after the gradual appearance of pathognomonic neurologic signs and symptoms. The lag in appearance of neuropathic findings relative to the onset of liver disease was most striking in patient 16, who presented with a Reye's syndrome–like episode at 5 months of age and underwent an evaluation for NN because he had a previously affected sibling. At 7 months of age, neurologic examination, electromyography, and sural nerve histologic findings were normal. Over the next 3 years, he developed typical neuropathic features of NN including peripheral weakness, loss of peripheral sensation, and corneal ulcers. At 4 years of age, repeat nerve conduc-

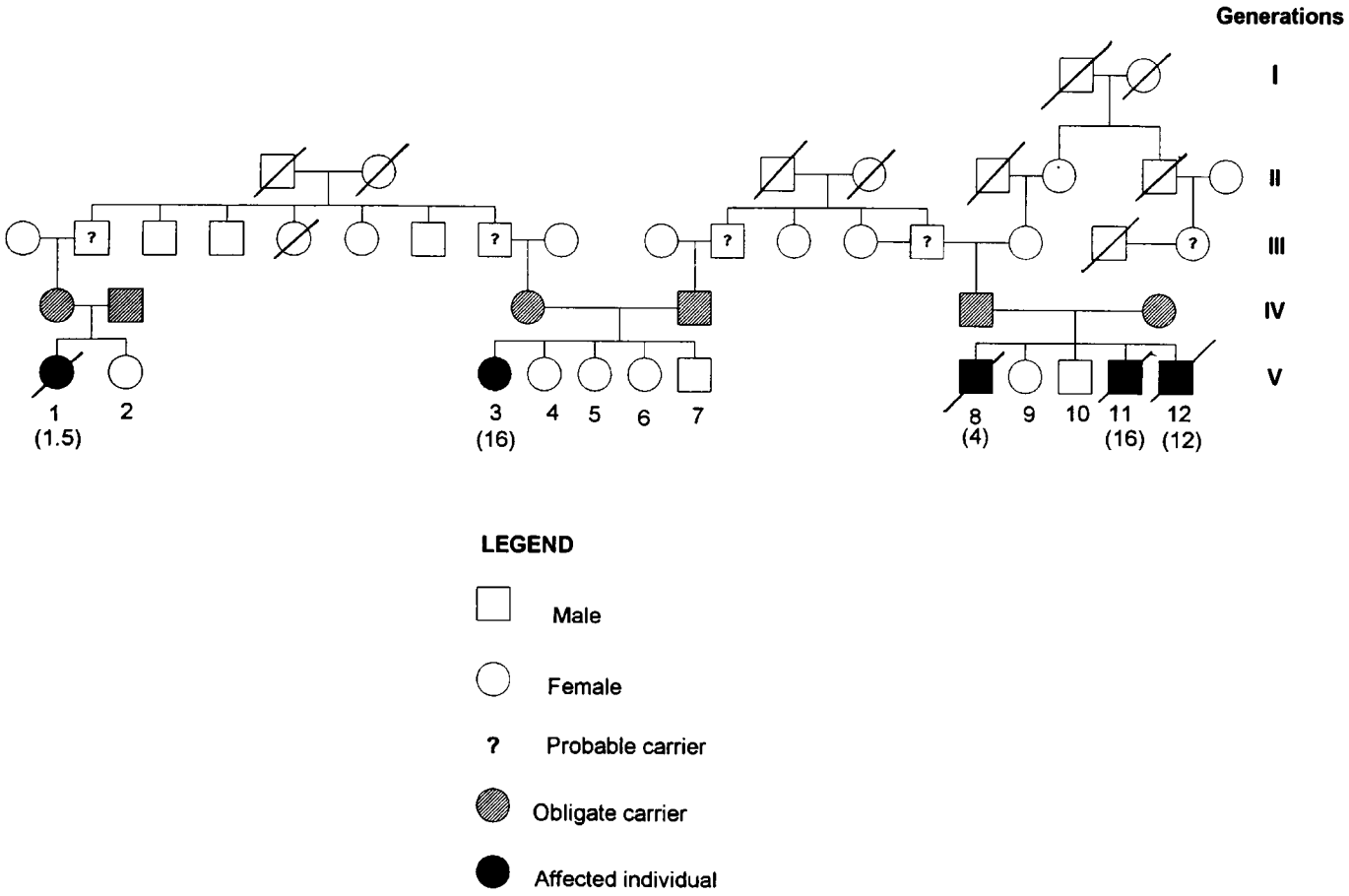

Fig 4. Pedigree of kindred with multiple family members with NN. Current age in years, or age at death in years, in parentheses under symbols. Patients V-3 (No. 12 in this report), V-11, and V-12 had classical NN. Patient V-8 had childhood NN. Patient V-1 (No. 5 in this report) had infantile NN. Patients V-8, V-11, and V-12 were born before the period of this study but were previously described by Singleton et al.[2]

tion velocity and electromyography were markedly abnormal and consistent with a diagnosis of NN.

Three of the 5 patients who presented with liver disease in infancy experienced progression to cirrhosis and 2 died, at 7 and 12 years of age. Patient 13 had cirrhosis and underwent liver transplantation. Of the 4 patients with non-hepatic presentations of NN (such as FTT or corneal ulcers), all are alive and only one has developed cirrhosis. The milder course of illness in these 4 patients suggests that lack of hepatic disease in infancy may be a more favorable prognostic sign.

Liver histologic findings from 6 of the patients were characterized by less striking findings than the other 2 forms of NN, although no liver specimen was normal. Chronic portal tract inflammation and advanced bridging fibrosis or cirrhosis were noted (Fig 2, *D*). Milder steatosis (primarily microvesicular) with less cholestasis, hepatocyte ballooning, and giant cell transformation was observed as compared with the infantile and childhood NN histology. Biopsy specimens from 2 patients showed mild to moderate bile duct proliferation associated with cirrhosis.

Ultrastructural Evaluation of Liver

Electron microscopic evaluation of liver specimens from 4 of the patients was undertaken at time of biopsy (Fig 3, *A* and *B*). Material was available from one patient with the infantile form of NN and 3 with the classical form of NN. All showed abnormalities of mitochondria (ringed cristae, swelling and loss of cristae, pleomorphic contour), effacement of canalicular microvilli, and accumulation of intracellular bile pigments and fat-containing vesicles. Peroxisomes were present and normal in appearance in all specimens. No other lysosomal storage material was observed.

Comparison of Liver Histologic Findings

Comparison of liver histologic findings among the 3 phenotypic groups showed that scores of individual features of the patients with infantile and childhood NN tended to be higher than those of the patients with classical NN (Table V), particularly for ballooning degeneration, pseudo-acinar formation, and giant cell transformation. Regression analysis confirmed a significant association of histologic features into 2 distinct clusters. Giant cell transformation, ballooning degeneration, intracel-

lular cholestasis, and portal tract inflammation were significantly ($P < .05$) associated with each other. This association suggests that bile retention may be involved in the primary pathogenesis of the liver lesion of NN, inasmuch as hepatocellular retention of bile acids has previously been associated with giant cell transformation in the neonatal liver[4] and ballooning degeneration of the liver at any age.[5] Macrovesicular steatosis, microvesicular steatosis, pseudo-acinar formation, and canalicular cholestasis were associated ($P < .05$) with each other. Hepatic fibrosis or cirrhosis was not significantly associated with any other specific histologic feature but was present in all histologic material. Many of these findings of the NN liver lesion (steatosis, pseudoacini, fibrosis, and cirrhosis) are consistent with a suspected metabolic etiology to this disorder.[6]

Pedigree Analysis

Pedigree analysis showed that all NN phenotypes could be found within an extended kindred and that different phenotypes were seen even within a single family (Fig 4). Of 3 affected brothers, born before this study period and described in the report by Singleton et al,[2] 2 had classical NN and 1 had childhood NN. The pedigree also shows a second cousin (V3 in Fig 4 and patient 12 in this study) with classical NN and another cousin (V1 in Fig 4 and patient 5 in this study) with infantile NN. Other families have shown siblings with classical NN and infantile NN (patients 1 and 17).

All 20 patients have ancestry from the western portion of the Navajo Reservation. As of the 1990 census, the population of the western Reservation was 60,000. Historical records show that the large majority of the population was descended from approximately 1000 Navajo who fled to this region to avoid capture or death at the hands of the US military during armed conflict in 1864.[8-10] Since the late 19th century, this area has had rapid population expansion with a fertility rate 4 times that of the United States.[11] The geographic isolation and high fertility rate of the western portion of the Navajo Reservation are thought to have contributed to the potential for a "founder effect" or genetic drift, as evidenced by 2 other rare, heritable illnesses among this subpopulation: metachromatic leukodystrophy[12] and severe combined immunodeficiency syndrome.[13] Within this geographically and historically defined subpopulation of the western Navajo Reservation, the incidence of NN is 1 in 1600 live births.

DISCUSSION

In this report we demonstrate the previously underappreciated extent of liver disease in patients with NN. We have shown that all children with NN have evidence of ongoing biochemical hepatic injury, including those without clinical evidence of liver disease, and that progression to liver failure or cirrhosis is the factor that determines survival in patients with NN. In addition, we extend the spectrum of disease in NN to include fatal liver disease in infancy and childhood.

The validity of establishing the diagnosis of NN in infants and young children primarily with liver disease might be questioned. Although these patients meet our diagnostic criteria for NN, most lack biopsy-proven demyelination, and all lack the striking features of corneal scarring and acral mutilation that are characteristic of older patients with NN. It could be argued that nutritional deficits (such as secondary vitamin E or vitamin A deficiencies) or other causes are responsible for the development of neurologic abnormalities in these patients with liver disease; however, we do not believe this to be the case. The neurologic deficits that developed in these patients are clearly different from those of secondary vitamin E deficiency, which is primarily a cerebellar ataxia.[14] The nerve biopsy findings are also not consistent, with loss of myelinated fibers being restricted to large-caliber fibers in vitamin E deficiency.[14] Furthermore, serum vitamin E concentrations and serum lipids were normal in 3 of 4 infants tested and in all older patients with NN. The corneal anesthesia in NN is not observed in vitamin A deficiency, although xerosis and corneal scarring may occur in the latter.[15] Serum retinol levels were normal in all patients with NN tested (data not shown). Finally, the striking pattern of demyelination observed by central nervous system imaging was unlike changes caused by chronic liver disease[16] but was characteristic of those reported in NN.[3,4] Thus there is no evidence that the neuropathic findings in our patients developed as a consequence of another chronic liver disease or from a nutritional deficiency.

Several factors argue in favor of the 3 groups of patients described in this report having phenotypic variants of a single disease rather than representing several different disorders. These include the presence of relatives with classic NN in the kindreds of patients with infantile and childhood NN, the exclusion of other known causes of liver disease, and the overlap of hepatic and neurologic symptoms at various ages in all patients. The identification of a definitive genetic or biochemical marker for NN would resolve this issue, but such a marker remains elusive at present. The only pathologic correlate recognized for NN is the original description of generalized loss of myelinated fibers on peripheral nerve biopsy specimens, described by Appenzeller et al.[1] In our series, 1 of 2 patients with infantile NN and 2 of 3 patients with childhood NN who underwent sural nerve biopsy showed typical loss of myelinated nerve fibers. In many of the younger patients nerve biopsy was not considered because the diagnosis of NN was not entertained before death. However, even if all infants had undergone sural nerve biopsy, it is likely that the typical lesion would have been absent in the younger patients because of

the gradual development of neuropathic findings and the sural nerve lesion.[1,2] The clearest demonstration of this is patient 16 in this report, who had normal findings on neurologic examination and on a sural nerve biopsy specimen at age 7 months (obtained because of the concern about NN as a cause of a Reye's syndrome–like episode and a positive family history of NN), followed by development of a progressive sensorimotor neuropathy and corneal ulcers typical of NN. Thus the diagnosis of NN, especially at a younger age, will remain a clinical, and not a pathologic, diagnosis.

Similarly, the absence of characteristic secondary neuropathic findings (acral mutilation or corneal ulceration) in infants and younger children does not exclude the diagnosis of NN. In patients with classical NN, the only neurologic signs and symptoms that developed before 2 years of age were gross motor delay, peripheral weakness, and areflexia. Mean age at onset in these patients was 3.2 years for corneal ulcers, 3.7 years for sensory neuropathy, and 4.6 years for acral mutilation (unpublished data). Thus we postulate that the patients with infantile and childhood NN would have eventually manifested these more characteristic neuropathic findings of NN if they had not died prematurely of liver disease. This is supported by the clinical course of patient 4 with infantile NN, who had no observed neurologic disease at 9 months of age when her death was prevented by liver transplantation. In subsequent years she has developed the sensorimotor neuropathy and corneal anesthesia that are typical of NN.

Although neuropathic disease seems to progress steadily in NN, it is not understood what environmental or other genetic factors control the appearance, resolution, or progression of hepatic disease in NN. A similar variability of hepatic disease has been described in other metabolic liver diseases, such as A1AT deficiency. Patients with A1AT deficiency and the *PiZZ* phenotype may have no liver disease, cholestasis in infancy that resolves, or progression of disease to cirrhosis and death.[17] Hepatotoxins, such as alcohol, or viral illnesses, such as hepatitis C, are associated with progression of liver disease in A1AT deficiency.[18] No similar aggravating factors have yet been identified in NN.

The etiology of NN remains unknown. The infantile form of NN shares a number of features associated with progressive familial intrahepatic cholestasis, a group of disorders caused by inborn errors in hepatic secretory processes. Patients with various forms of PFIC present in infancy with FTT, cholestasis, progressive cirrhosis, and liver failure. Unlike patients with NN, they have no known neurologic disease (except that secondary to vitamin E deficiency) and often have severe pruritus.[19-21] Patients with PFIC-1 (Byler's disease; mutations of FIC-1)[22] and PFIC-2 (mutations of the bile salt export pump gene)[23] have normal serum GGT concentrations, which are elevated in nearly all patients with NN. At the microscopic level, liver histologic findings in NN include prominent microvesicular and macrovesicular steatosis, which is absent in PFIC.[22] Furthermore, the characteristic ultrastructural feature of canalicular granular bile in PFIC-1[22] was absent in the liver biopsy specimens from patients with NN. Patients with the newly described inherited defect in the multidrug-resistance 3 *(MDR3)* gene[20,24] have elevated serum GGT, as did many of the patients with NN. However, in *MDR3* deficiency neurologic dysfunction has not been reported, and liver histology did not show steatosis.[20,24] Nevertheless, preliminary findings suggest that *MDR3* expression may be low in the liver of patients with NN.[25] This observation will require confirmation and comparison with liver from unaffected Navajos, as well as with liver from similarly aged Navajos with other cirrhotic disorders, and genetic analysis.

The childhood form of NN has a number of clinical similarities with Alpers' disease, a mitochondrial disorder involving complex I of the respiratory chain.[26] This disease presents in early childhood with FTT, hypotonia, and seizures and progresses to liver failure. Although not identical, similar multi-system involvement and age at onset in NN have led to the proposal that NN may also be a mitochondrial disorder.[27-29] The elevated serum lactate levels observed in a number of patients with NN fit with this hypothesis. Results of skin fibroblast respiration studies were normal in 3 patients with NN in this report; however, normal fibroblast respiratory enzyme activity does not preclude the presence of mitochondrial pathology in other tissues such as the liver or nervous system. Finally, the possibility of a disturbance of fatty acid oxidation has been considered, although results of investigations have been negative. Serum acylcarnitine profiles were normal in the 3 patients tested, urine organic acid levels were normal in the 9 patients with childhood and classic NN tested, and serum carnitine and free carnitine levels were normal in the 3 patients tested. The mildly elevated levels of dicarboxylic acids in the urine of the 3 with patients infantile NN were believed to be consistent with the presence of severe liver disease. In addition, one of the patients with infantile NN showed normal oxidation of palmitic acid and myristic acid by cultured skin fibroblasts and normal carnitine palmitoyl transferase activities.

Definitive diagnosis of NN will be problematic until a specific biochemical or genetic marker is identified. This is especially true for infants and children who present with liver disease, because current diagnostic criteria depend on neurologic findings, which do not appear until an older age. We propose adopting the diagnostic criteria used in this study, which would take into account affected relatives with NN. The criteria would also include central nervous system imaging findings and corneal anesthesia, which ap-

pear at earlier ages than sensory neuropathy or corneal ulceration. Navajo infants or young children who present with unexplained liver disease should have the diagnosis of NN considered and undergo a careful neurologic evaluation including cranial imaging, electromyography, and sural nerve biopsy. Current treatment options are limited by the lack of understanding of the underlying pathogenesis of NN. Caution should be entertained in performing liver transplantation for patients with suspected NN because of the possible development of progressive neurologic dysfunction after transplantation.

We conclude that overlap of hepatic and neurologic findings in individual patients, the presence of different phenotypes in a single kindred, and the historical genetic isolation of the Navajo population support the proposal that NN is a single genotype with varied phenotypic expression. The identification of a genetic marker for this disease and the development of an effective treatment would be of great benefit to the Navajo people. Because of the uniform involvement of the liver in all patients with this disorder, we propose that the name of this disease be changed to *Navajo neurohepatopathy.*

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