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Morphologic Findings in Progressive Familial Intrahepatic Cholestasis 2 (PFIC2): Correlation With Genetic and Immunohistochemical Studies

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Abstract

Progressive familial intrahepatic cholestasis, type 2 (PFIC2), characterized by cholestasis in infancy that may progress to cirrhosis, is caused by mutation in *ABCB11*, which encodes bile salt export pump (BSEP). We correlated histopathologic, immunohistochemical, and ultrastructural features in PFIC2 with specific mutations and clinical course. Twelve patients with clinical PFIC2 and *ABCB11* mutations were identified, and 22 liver biopsy and explant specimens were assessed. All had hepatocellular cholestasis; most had canalicular bile plugs. At least 1 specimen from every patient had centrizonal/sinusoidal fibrosis, often with periportal fibrosis. Neonatal hepatitis-like features (inflammation, giant cells, necrosis) varied. In 2 of the 5 patients with paired specimens obtained > 6 months apart, lobular and portal fibrosis worsened. Transmission electron microscopy (EM) in all 9 patients studied showed canalicular dilatation, microvilli loss, abnormal mitochondrial internal structure, and varying intra-canalicular accumulation of finely granular bile. Canalicular staining for BSEP was absent in 10 patients and present in 2 patients, 1 of whom had intermittent symptoms. *ABCB11* sequencing of all patients identified 6 novel and 10 previously described mutations, with nonsense, missense, and/or noncoding mutations in the 10 patients without immunohistochemically demonstrable BSEP. Missense and/or noncoding mutations were identified in the 2 patients with demonstrable BSEP, whose clinical course was more indolent. Mutations ending *ABCB11* transcription appear linked, through hepatocellular necrosis and fibrosis, to worse outcome. In conclusion, light microscopy and electron microscopy findings in clinical PFIC2 can support diagnosis, but are variable and nonspecific. Therefore, no correlation between specific mutations and histopathology is yet possible.

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Keywords

PFIC2; progressive familial intrahepatic cholestasis; bile salt export pump; BSEP; *ABCB11*; neonatal hepatitis

Progressive familial intrahepatic cholestasis, type 2 (PFIC2) is a rare form of cholestasis in infancy that results from mutations in *ABCB11*, which encodes bile salt export pump (BSEP). Specific mutations in *ABCB11* are related to BSEP expression and clinical features. The classically described histology of early PFIC2 includes neonatal hepatitis-like changes such as giant cell transformation, inflammation, and canalicular cholestasis.[3] Later changes include canalicular cholestasis, ductular reaction or proliferation, and fibrosis.[5]

In this study, we correlate morphologic and ultra-structural features of the liver with clinical and genetic findings in patients with genetically confirmed PFIC2. We also describe the histologic progression of PFIC2 liver disease in a subset of patients who had multiple liver tissue evaluations.

MATERIALS AND METHODS

Twelve patients (7 boys, 5 girls) with clinical PFIC2 and mutations in *ABCB11* were identified by review of hospital records. All patients were infants at the time of symptom onset, and had normal or slightly elevated serum γ-glutamyltransferase values at presentation despite nonremitting conjugated hyperbilirubinemia. All patients had mutation(s) in *ABCB11*. Coding sequence, including intron-exon junctions, was evaluated by polymerase chain reaction amplification and sequencing of the exons and flanking intron regions of the gene, performed commercially for patients 9–11 (Baylor College of Medicine, Houston, TX) or by customized cholestasis chip analysis.[11,13] In patient 12, whose sister had been diagnosed with PFIC2, only regions abnormal in his sister were sequenced; in all other patients, the entire gene was examined. Liver biopsy and/or explant specimens, obtained between 1995 and 2010, were drawn from the files of Cincinnati Children's Hospital Medical Center, Cincinnati, OH, Texas Children's Hospital, Houston, TX, and University of California, San Francisco, CA. Specimens from all patients were retrospectively assessed by GK and KE. Hematoxylin/eosin-stained and trichrome-stained sections of formalin-fixed, paraffin-embedded tissue were reviewed.

The extent of lobular mononuclear leukocyte inflammation was scored as absent, rare (<1 focus per lobule), focal (≥ 1 focus per lobule), or diffuse (generalized, present in most lobules). The extent of hepatocellular necrosis was scored as absent/rare (≤2 necrotic hepatocytes per sample), few (>2 scattered necrotic hepatocytes per sample but ≤2 per lobule), or many (>2 scattered necrotic hepatocytes per lobule). Giant cells, defined as hepatocytes with 3 or more nuclei independent of cell size, were scored as absent, rare (1 or 2 per sample), present (giant cells occupying up to 10% of lobular area), common (giant cells occupying 10% to 49% of lobular area), or prominent (giant cells occupying ≥ 50% of lobular area). Hepatocellular swelling, defined as cellular enlargement with rarefaction of cytoplasm in the absence of distinct vacuoles, was scored as absent, rare (<5% of lobular area), present (5% to <50% of lobular area), or prominent (50% or more of lobular area). Pseudorosette formation, defined as a dilated canaliculus bordered by >2 hepatocytes, was scored as absent, present (<1 per lobule), or prominent (at least 1 in every lobule). Lobular sinusoidal/centrizonal fibrosis was graded as absent, focal (<50% of lobular area), or prominent (≥ 50% of lobular area), and if present, was assessed for extension beyond zone 3.

The intensity of portal mononuclear leukocyte inflammation was scored as absent, focal (<50% of portal areas), or generalized (≥ 50% of portal areas), and if present, was graded as mild or moderate/marked. Interface ductular reaction was scored as absent, focal (< 50% of portal areas), or generalized (≥50% of portal areas), and if present, was graded as mild or moderate to severe. The distribution of bile plugs, if present, was scored as canalicular and/or duct/ductular. Bile ducts were examined for injury (nuclear size variation, vacuolated cytoplasm, and/or apoptosis). Portal fibrosis was scored by the Scheuer criteria,[18] with stage 0 indicating no fibrosis, stage 1 indicating fibrous portal tract expansion, stage 2 indicating periportal or portal-portal septa with an intact architecture, stage 3 indicating bridging fibrosis with distorted architecture, and stage 4 indicating probable or definite cirrhosis.

Immunostaining for BSEP, with a homologous canalicular transporter control, multidrug resistance-associated protein 2 (MRP2, *ABCC2*), was performed as reported[7] for clinical diagnostic purposes on at least 1 liver sample from all patients. The polyclonal anti-BSEP antibody used, raised in rabbit, was a generous gift from Dr B. Stieger. Sections were heated for 10 minutes in ethylenediamine tetraacetic acid-Tris buffer (pH 9); the antibody was used at 1:500 dilution of the original stock. Immunostaining for BSEP was scored as present, defined as positive canalicular staining, or absent. Transmission electron microscopy (EM) was performed and evaluated in 9 patients (9 samples). Clinical and laboratory findings were studied by examination of medical records. This study was approved by the Institutional Review Board of the University of California San Francisco, Cincinnati Children's Hospital, and Texas Children's Hospital.

RESULTS

Clinical and Laboratory Features

Patients ranged from 1 to 8 months of age at sign or symptom onset; initial signs and symptoms included jaundice, pruritus, poor weight gain, bruising, and bleeding (Table 1). Patients ranged from 6 weeks to 7 years of age on first evaluation at our institution. In 11 cases, this was the patient's first comprehensive evaluation for liver disease. Patient 9, the older brother of patient 10, had been previously evaluated in Saudi Arabia, and these early records were not available for our study. Serum transaminase activity and γ-glutamyltransferase activity were elevated and normal or mildly elevated, respectively, in all patients. Almost all patients presented with pruritus and/or jaundice; patient 11 presented with intracranial bleeding, and patient 12 came to clinical attention because of an affected older sister (patient 2). Clinical illness was intermittent in 1 patient (patient 7).

Three patients were treated for pruritus with ileal exclusion at the ages of 13 months, 15 months, and 9 years. The youngest of these patients experienced continued pruritus and was transplanted at the age of 18 months (Table 1). The other 2 patients responded to treatment. Three patients were treated for pruritus with external biliary diversion at ages of 11 months, 17 months, and 22 months. The youngest of these patients was later transplanted at the age of 16 months because of progressive coagulopathy, portal hypertension, and ascites. The other 2 patients responded to treatment.

In addition to the 2 patients who received liver transplants after failing to respond to surgical intervention, 5 other patients were transplanted, at ages ranging from 17 months to 7 years. Five patients (42%) survived with their native liver with a median follow-up of 41 months (range, 23 to 180 mo). One patient had not undergone any surgical intervention by the last follow-up (age, 30 mo). All patients were alive at the last follow-up.

Histopathologic Features

Fifteen liver biopsy specimens and 7 explanted livers were available for study (22 samples total). Lobular and portal tract changes are summarized in Tables 2 and 3. The amount of lobular inflammation varied greatly, ranging from absent or rare (2 and 7 samples, respectively) to diffuse (5 samples) (Table 3, Figs. 1A, C, E and Figs. 2A, C). Similarly, the number of necrotic hepatocytes varied. They were absent/rare in 14 samples, few in 4 samples, and many in 4 samples, all of which were from patients who underwent transplantation (patients 4, 6, and 12). Portal tract inflammation also varied. Giant cell transformation was present at least rarely in all 22 samples, and was prominent in 4 of 6 samples obtained during infancy (<12 mo of age).

All samples showed hepatocellular cholestasis. Most samples (19 samples) had bile plugs in canaliculi; 2 of these also had bile plugs in ductules and, rarely, in small ducts. Pseudorosette formation was either present (9 samples) or prominent (11 samples) in most specimens. Hepatocellular swelling was variable, but 19 samples showed at least some swelling, and prominent swelling was observed in 9 samples. Interlobular bile duct injury was noted in only 1 needle biopsy specimen and was mild. No sample had mononuclear inflammatory cells infiltrating the biliary epithelium or within the duct lumina. No portal tract edema, periductal fibrosis, or acute cholangitis was observed in any sample.

All patients showed centrizonal/sinusoidal fibrosis in at least 1 sample (Table 2, Figs. 1B, D, F and Figs. 2B, D). This pericellular fibrosis was usually prominent (16 samples) and often extended beyond zone 3 (14 samples) (Fig. 1F). Similarly, portal fibrosis was common and often prominent, particularly in the 7 patients (patients 2, 4, 5, 6, 9, 11, and 12) who required liver transplantation (Table 2, Fig. 1D). Of the 9 samples from the patients who survived with their native livers (patients 1, 3, 7, 8, and 10), only 1 showed more than stage 2 portal fibrosis.

After their initial biopsies, 5 patients underwent at least 1 additional procedure (biopsy or transplant) >6 months later. Two of these patients (patients 6 and 8) showed progression of both lobular and portal fibrosis over time. No trichrome-stained section of the initial biopsy specimen from patient 4 was available; thus, the progression of fibrosis could not be assessed. Patient 11 had advanced fibrosis by his initial biopsy at the age of 3 years, and his explant at the age of 5 years showed the same stage of fibrosis. In patient 7, multiple liver biopsies showed stable liver disease with exacerbations of lobular cholestasis and hepatitis (Figs. 2A–D).

Immunohistochemical Staining

Most patients (10 of 12) lacked detectable BSEP (Fig. 2F, Table 4). Canalicular staining for BSEP was observed in patients 3 and 7; the latter is our oldest patient (age 15 years at the last follow-up) (Fig. 2E). Canalicular expression of MRP2 (not shown) was present in all patients, indicating that when BSEP marking was lost, this was a specific disease-related phenomenon.

Transmission EM

Ultrastructural abnormalities in the 9 patients studied were similar to each other and to those reported in patients with PFIC2,[3] serving to confirm the changes observed by light microscopy and differing among each other only in degree. The major findings were nonspecific features of lobular cholestasis such as increased dense polymorphous secondary lysosomes, canalicular dilatation with effacement of microvilli, variable accumulation of finely granular bile in canalicular lumina (Fig. 3A), and subtle nonspecific abnormalities of mitochondrial internal structure (Fig. 3B). In this series of patients, the bile never resembled

the coarsely particulate bile characteristic of PFIC1.[3] A circumferential band of intermediate filaments, presumed to be actin, was often visible around dilated canaliculi and was present as early as 12 months of age in patient 3.

Genetics

Sequencing showed 12 different single nucleotide substitutions in the coding region and 4 in the noncoding region of *ABCB11* in the 12 patients. Of the former, 3 variants are predicted to result in nonsense mutations and 9 in missense mutations. Ten mutations have been described before[5,7,10,13,16,19,20]; the new variants include 4 sequence alterations predicted to cause deleterious mis-sense mutations (Table 4). For 11 patients, mutations in *ABCB11* were identified on both alleles. Only 1 mutation was identified in patient 8; this mutation was most likely heterozygous due to a lack of a history of consanguinity. (The other allele in this patient may contain a mutation located outside the sequenced region or a microdeletion.) Nonsense, missense, or noncoding region mutations were identified in the 10 patients without immunohistochemically demonstrable BSEP. Missense and/or noncoding region mutations were identified in the 2 patients with immunohistochemically demonstrable BSEP.

DISCUSSION

Here we describe the morphologic, immunohistochemical, and ultrastructural features in 12 patients with genetically confirmed PFIC2. All liver samples showed lobular cholestasis, and most had bile plugs in canaliculi. Other common features included lobular and/or portal inflammation, giant cell transformation, hepatocellular necrosis, and hepatocellular swelling. This neonatal hepatitis-like pattern has been described in PFIC2, particularly in early biopsy specimens.[1,4,6,12,14,15,21] A comprehensive histopathologic examination of 84 liver biopsy specimens in the setting of PFIC identified similar features in some specimens, including hepatocellular and canalicular cholestasis, ballooned hepatocytes, and giant cell transformation, and prominent bile duct loss and injury,[2] but this study was carried out before the genetic basis of PFIC2 was known.[19] Thus, it may have included cases of PFIC1, a related form of cholestatic liver disease caused by mutations in familial intrahepatic cholestasis, type 1 (*FIC1*)/*ATP8B1* that clinically can resemble PFIC2.[9] The bile duct loss and injury reported[2] might have been observed in patients with PFIC1, as these changes were not characteristic in our patients with PFIC2.

A comparison of histopathologic differences between PFIC1 and PFIC2 reported portal and lobular fibrosis, many giant hepatocytes, and occasional hepatocyte necrosis in PFIC2, just as we have observed.[5] Chart review of patients with PFIC1 and patients with PFIC2 also identified giant or multinucleate hepatocytes in most patients with PFIC2,[16] whereas giant hepatocytes and necrosis were rare in PFIC1.[5,16] In our study, giant cells were present at least rarely in all samples, and were prominent in most samples obtained during infancy (<12 mo of age). Thus, our observations are in keeping with the major histologic findings of these 2 recent studies, and support the proposition that giant cells (even in patients older than 1 y), portal and lobular fibrosis, and occasional hepatocyte necrosis characterize PFIC2.

Our study included 5 patients with multiple liver samples taken at least 6 months apart, which let us examine changes in histopathologic features of PFIC2 over time (Table 2). Interestingly, the prominence of neonatal hepatitis-like findings varied greatly among patients and within samples from individual patients, and we observed no clear trends. For example, in 3 patients, the frequency of giant cells remained approximately the same over time, whereas in 2 patients the frequency decreased. Lobular inflammation fluctuated in 2 patients and remained similar in 3 patients. Hepatocellular swelling fluctuated in 1 patient, remained similar in 3 patients, and decreased in 1 patient. However, our finding that giant

cells and lobular inflammation were either rare or absent in some biopsy specimens indicates that the absence of classic neonatal hepatitis-like features on light microscopy does not exclude the diagnosis of PFIC2. Importantly, biopsy specimens from 3 of our oldest patients (patients 7, 9, and 11; ages 9, 7, and 5 y, respectively) continued to show at least some neonatal hepatitis-like features, including giant cells, hepatocyte swelling, and inflammation. This finding indicates that regardless of age, patients with PFIC2 are likely to show at least some neonatal hepatitis-like changes on biopsy.

All patients had some portal-based fibrosis in at least 1 sample. Variation in portal fibrosis, including progression to micronodular cirrhosis, has been reported in PFIC2.[4,5,10,12,14–17,20,21] All patients in this study had sinusoidal/centrizonal fibrosis in at least 1 sample. Sinusoidal fibrosis was usually prominent, and often extended beyond zone 3. The pronounced lobular fibrosis in PFIC2 is distinct from portal-based or post-necrotic fibrosis. Lobular fibrosis, together with lobular inflammation, giant cells, and cell swelling, may be helpful clues to the possibility of PFIC2 on initial light microscopic examination in the appropriate clinical setting, and should perhaps prompt immunohistochemical and genetic studies.

Mutations in *ABCB11* are typically associated with loss of immunohistochemically demonstrable BSEP expression, indicating that immunohistochemical staining is a useful diagnostic tool in PFIC2.[5,7,10,14,15,17,20,21] In our study, 10 patients lacked BSEP expression on immunohistochemical analysis. However, patients with clinical PFIC2 and mutations in *ABCB11* may still have immunohistochemically detectable BSEP[20]; in such patients the protein expressed may be quantitatively insufficient or not fully functional. Patients 3 and 7 expressed BSEP on immunohistochemical analysis. Both had missense mutations (p.G116E and p.V1212F) predicted to affect the highly conserved nucleotide-binding folds of BSEP. These results are in keeping with the findings of Strautnieks et al,[20] who detected BSEP expression in 28% of patients with PFIC2, most of whom had missense mutations within the nucleotide-binding folds.

An interesting question is whether the presence of intact BSEP expression correlates with clinical and/or histologic findings. The 2 BSEP-expressing patients in this study have followed a relatively slow progressive clinical course. Patient 7 was treated with ileal exclusion at 9 years of age and has not required additional surgical intervention. Patient 3 has not yet required surgical intervention till age 30 months. All biopsy specimens from these 2 patients showed only minimal or mild portal fibrosis (stages 1 or 2). These findings suggest that in PFIC2, patients with intact BSEP expression tend to develop less portal fibrosis than patients without BSEP expression, and follow a correspondingly more indolent clinical course. However, sinusoidal fibrosis, inflammatory features, and cholestatic changes in these BSEP-expressing patients varied, and did not substantially differ from those in patients without BSEP expression.

A related issue is whether specific genetic mutations are correlated with clinical and/or histologic findings. In this study, we found 6 *ABCB11* mutations associated with clinical PFIC2 that have not been previously published, and identified 10 mutations that were previously published (Table 4).[5,7,10,13,16,19,20] The diversity of *ABCB11* mutations and the wide spectrum of findings on light microscopy, particularly the variations in samples from individual patients over time, indicate that severity of inflammatory and/or cholestatic features cannot be correlated with specific genetic mutations. Perhaps some of the variation reflects the realities of tissue sampling in a heterogeneous setting or reflects intercurrent, non-PFIC2-related hepatic injury. In a large study of patients with PFIC2, Strautnieks et al[20] found that patients with 2 predicted protein-truncating mutations were at increased risk of developing hepatobiliary malignancy compared with other patients with PFIC2. That study

raised the possibility that certain types of genetic mutations in PFIC2 may be correlated with clinical outcomes. A study of 180 families with PFIC1 or benign recurrent intra-hepatic cholestasis, type 1 (BRIC1), both caused by mutation in *ATP8B1*, correlated mutation type/location with clinical severity.[8] An analogous study for *ABCB11*, correlating clinical presentations of PFIC2 and BRIC2 with mutation class, has not yet been conducted. Descriptive analysis in our small sample suggests that mutations predicted to abolish *ABCB11* transcription are linked through lost BSEP expression, by more necrotic hepatocytes and more fibrosis, to worse outcome. Assessment in larger cohorts may validate this impression.

In summary, key light microscopy findings in PFIC2 include cholestasis, lobular fibrosis, lobular inflammation, portal inflammation, portal fibrosis, giant cell transformation, hepatocellular necrosis, and hepatocellular swelling. EM shows canalicular dilatation, partial-to-complete loss of microvilli, abnormal mitochondrial internal structure, and varying accumulation of finely granular bile. Our study underscores the variation in histologic findings that may be seen in PFIC2, both among patients and within the same patient over time. Light microscopic features are not clearly associated with clinical course in patients with PFIC2. Thus, although morphologic features can provide some support for the diagnosis of PFIC2, immunohistochemical or genetic studies, sometimes in combination, are required to establish the diagnosis.

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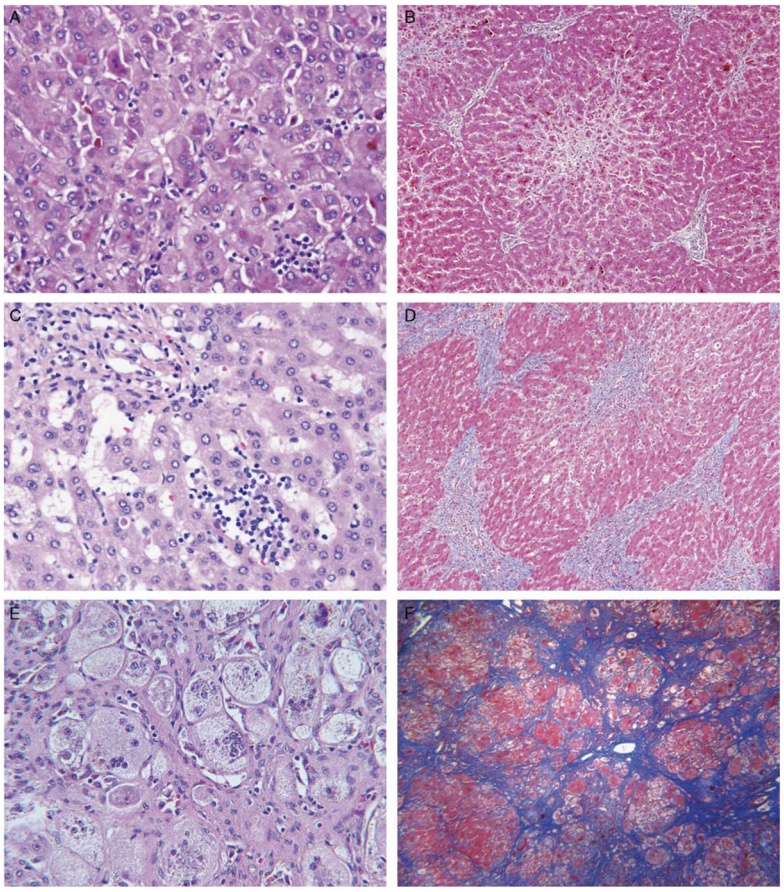

FIGURE 1.

Light microscopy findings in PFIC2 include varying lobular inflammation, portal inflammation, giant cell transformation, hepatocellular necrosis, hepatocellular swelling, portal fibrosis, and centrizonal/sinusoidal fibrosis. A and B, Explanted liver of patient 12 at the age of 17 months shows cholestasis, focal lobular inflammation, hepatocellular necrosis (including necrotic giant cells), prominent centrizonal fibrosis, and stage 2 portal fibrosis. Hematoxylin and eosin (H&E; × 400) and trichrome (× 100) stains. C and D, Explanted liver of patient 9 at the age of 7 years shows more prominent, diffuse lobular inflammation and areas of bridging fibrosis (shown) and nodule formation (not shown) with architectural distortion. H&E (× 400) and trichrome (× 100) stains. E and F, Explanted liver of patient 6 at the age of 16 months shows giant cells, hepatocyte swelling, and centrizonal/sinusoidal fibrosis, all prominent, and cirrhosis. Patchy intense lobular inflammation and pervasive bands of vascularized intralobular replacement fibrosis are seen. Central veins and portal-to-portal scars are not discernible; most of the fibrosis is pericellular. H&E (× 160) and trichrome (× 100) stains.

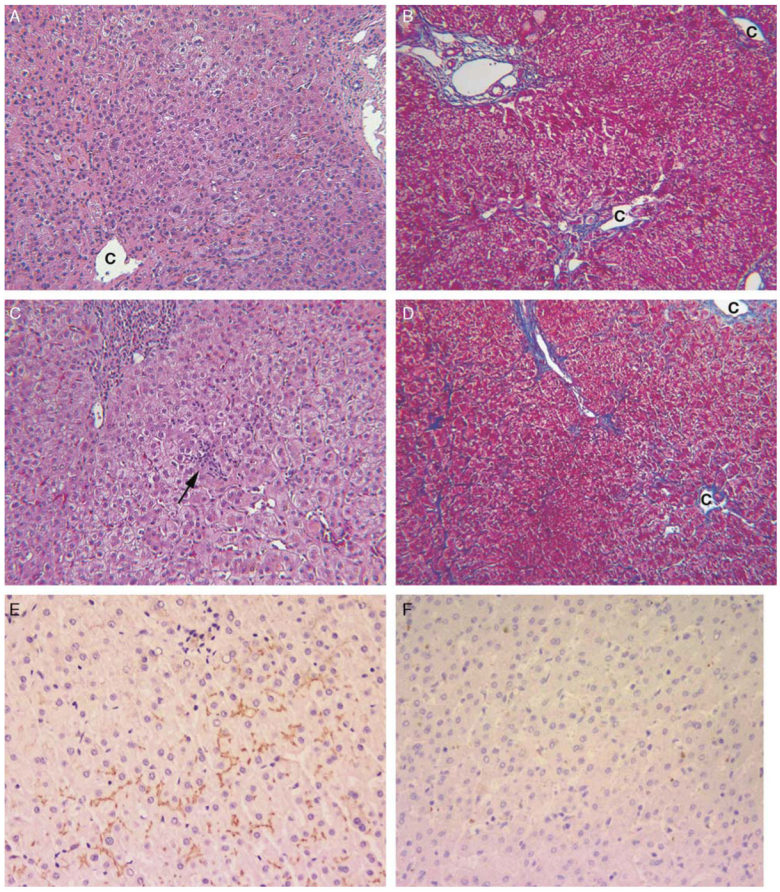

FIGURE 2.

Patient 7 had early-onset, intermittent pruritus and a long course (present age, 15 y), and his liver tissue showed intact canalicular BSEP immunostaining. Multiple liver biopsy specimens (A to D) showed stable liver disease with exacerbations of lobular cholestasis and hepatitis. A and B, Minimal cholestasis, occasional multinucleate giant hepatocytes, absent portal tract inflammation, and centrilobular fibrosis confined to zone 3 at the age of 13 months. C indicates central vein. Hematoxylin and eosin (H&E) and trichrome stains (each × 100). C and D, Mild portal and focal lobular inflammation (arrow) accompany lobular cholestasis and mild cell swelling at the age of 9 years. Fibrosis is mild. H&E and trichrome stains (each × 100) C indicates central vein. E, Immunostain for BSEP (hematoxylin counterstain) performed on liver biopsy tissue of patient 7 at the age of 9 years shows normal intensity and distribution of canalicular reactivity (× 200). F, In contrast, liver biopsy of patient 10 at the age of 21 months shows no canalicular reactivity. Immunostain for BSEP, hematoxylin counterstain (× 200).

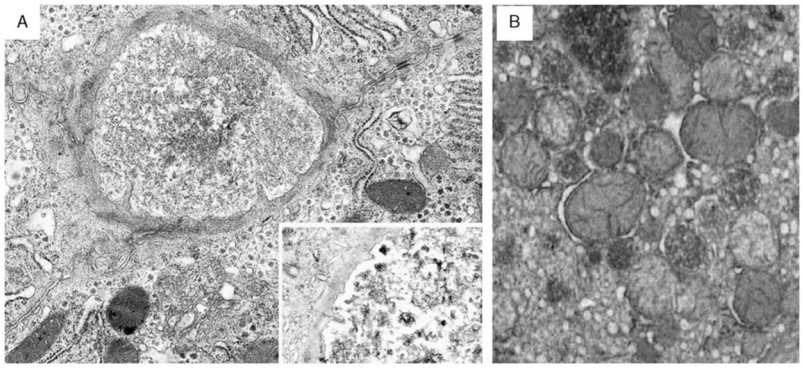

FIGURE 3.

Ultrastructural abnormalities of liver tissue were similar in the 9 patients studied. A, Liver biopsy from patient 7 at the age of 13 months shows canalicular dilatation, subtotal effacement of microvilli, a pericanalicular collar of microfilaments, and intact desmosomes. Canalicular bile is finely granular. Inset: Another severely dilated canaliculus in this patient contains occasional coarse aggregates mixed with the finely granular bile. Osmium tetroxide/uranyl acetate/lead citrate stain (× 3150). B, Liver biopsy from patient 11 at the age of 3 years shows mild mitochondrial pleomorphism, with alterations in size, shape, and matrix density. Osmium tetroxide/uranyl acetate/lead citrate stain (× 2000).

TABLE 1

Clinical and Laboratory Features

Patient	Age at Onset of Signs or Symptoms	Initial Symptoms and Signs	Age at Presentation for Medical Care	Symptoms and Signs at Presentation for Medical Care	Clinical-laboratory Results at Presentation*		Procedure(s); age	Indication	Age at the Last Follow-up
					GGT	ALT			
1	16 wk	Jaundice, hepatomegaly, bruising	5 mo	Pruritus, hepatomegaly	14	236	Biliary diversion, 17 mo	Pruritus	41 mo
2	6 mo	Jaundice, pruritus	17 mo	Pruritus	14	145	Ileal exclusion, 13 mo Transplant, 28 mo	Pruritus Continued pruritus	86 mo
3	ND	ND	9 mo	Jaundice, pruritus	14	93	None	NA	30 mo
4	8 wk	Jaundice	8 wk	Jaundice, scleral icterus	39	569	Transplant, 20 mo	Cirrhosis, pruritus	92 mo
5	ND	ND	30 mo	Jaundice, diarrhea	58	199	Transplant, 37 mo	ND	37 mo
6†	1 mo	Jaundice	6 mo	Jaundice, pruritus, failure to thrive, hepatomegaly, scleral icterus	35	959	Biliary diversion, 11 mo Transplant, 16 mo	Pruritus Portal hypertension, coagulopathy, ascites	3 y
7	6 wk	Poor weight gain	13 mo	Intermittent jaundice, intermittent pruritus, hepatosplenomegaly	35	60	Ileal exclusion, 9 y	Pruritus	15 y
8	3 mo	Pruritus	8 mo	Jaundice, pruritus, hepatomegaly, scleral icterus	29	254	Ileal exclusion, 15 mo	Pruritus	65 mo
9	2 mo	Jaundice, scleral icterus	7 y	Pruritus, poor growth, intermittent abdominal pain and distention	19	130	Transplant, 88 mo	Cirrhosis	92 mo
10	8 mo	Jaundice	21 mo	Jaundice, pruritus, poor growth, hepatosplenomegaly	24	164	Biliary diversion, 22 mo	Pruritus	23 mo
11	4 mo	Intracranial hemorrhage	4 mo	Coagulation abnormalities, mild hepatomegaly	19	285	Transplant, 62 mo	Pruritus	70 mo
12	3 mo	Jaundice, pruritus	6 wk	None (affected sibling)	33	259	Transplant, 17 mo	Pruritus	18 mo

*Expected ranges for these analytes are not provided because they were measured at different institutions and at different times.

†Patient 6 was included in a recent report of gene mutations in children with cholestasis.[13]

ALT indicates alanine transaminase; GGT, γ-glutamyltransferase; NA indicates not applicable; ND, not determined (unknown).

TABLE 2

Features

Type	Lobular Inflammation	Necrotic Hepatocytes	Giant Cells	Swelling	Pseudorosettes	Sinusoidal Fibrosis*	Portal-tract Inflammation	Ductular Reaction	Bile Plugs	Portal Fibrosis†
B	Rare	Few	Common	Present	Prominent	1	Focal, mild	Absent	Canalicular	1
E	Rare	Absent/rare	Present	Rare	Present	2	Focal, mild	Absent	Canalicular	2
B	Rare	Absent/rare	Rare	Absent	Present	1	Focal, mild	Absent	Absent	1
B	Focal	Absent/rare	Prominent	Prominent	Absent	ND	Generalized, moderate to severe	Absent	Absent	ND
E	Focal	Many	Present	Prominent	Present	4	Generalized, moderate to severe	Generalized, mild	Canalicular and ductal/ductular	4
E	Absent	Few	Rare	Prominent	Present	4	Focal, mild	Generalized, mild	Canalicular and ductal/ductular	4
B	Focal	Many	Prominent	Prominent	Prominent	0	Generalized, mild	Absent	Canalicular	0
B	Absent	Absent/rare	Prominent	Prominent	Prominent	4	Focal, mild	Absent	Canalicular	2
B	Diffuse	Many	Prominent	Prominent	Prominent	4	Generalized, mild	Absent	Canalicular	3
E	Focal	Absent/rare	Prominent	Prominent	Prominent	4	Generalized, mild	Absent	Absent	4
B	Focal	Absent/rare	Present	Present	Present	3	Absent	Focal, mild	Canalicular	2
B	Focal	Absent/rare	Rare	Prominent	Prominent	3	Generalized, moderate to severe	Focal, mild	Canalicular	1
B	Rare	Few	Rare	Rare	Absent	3	Generalized, mild	Generalized, mild	Canalicular	1
B	Focal	Absent/rare	Rare	Present	Present	4	Generalized, mild Generalized, mild	Absent	Canalicular	1
B	Rare	Absent/rare	Present	Prominent	Prominent	3	Focal, mild	Focal, mild	Canalicular	2
B	Rare	Few	Present	Rare	Present	4	Absent	Absent	Canalicular	3
B	Rare	Absent/rare	Rare	Rare	Prominent	2	Focal, mild	Absent	Canalicular	4
E	Diffuse	Absent/rare	Present	Rare	Present	4	Generalized, moderate to severe	Focal, mild	Canalicular	4
B	Diffuse	Absent/rare	Present	Rare	Present	4	Generalized, mild	Absent	Canalicular	1
B	Diffuse	Absent/rare	Present	Absent	Prominent	4	Generalized, mild	Focal, mild	Canalicular	3
E	Diffuse	Absent/rare	Present	Absent	Prominent	4	Generalized, mild	Absent	Canalicular	3
E	Focal	Many	Present	Present	Prominent	4	Focal, mild	Focal, mild	Canalicular	2

0, absent; 1, focal, confined to zone 3; 2, focal, extends beyond zone 3; 3, prominent, confined to zone 3; 4, prominent, extends beyond zone 3; ND, not determined (trichrome-stained
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ed by Scheuer criteria.

ecimen; E, explanted liver.

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TABLE 3

Frequency of Major Morphologic Features

Feature		No. (%)
Lobular sinusoidal fibrosis	Prominent, extends beyond zone 3	12 (57)
	Prominent, confined to zone 3	4 (19)
	Focal, extends beyond zone 3	2 (10)
	Focal, confined to zone 3	2 (10)
	Absent	1 (5)
	Cannot assess	1
Portal fibrosis (Scheuer criteria)	Stage 4	5 (24)
	Stage 3	4 (19)
	Stage 2	5 (24)
	Stage 1	6 (29)
	Stage 0	1 (5)
	Cannot assess	1
Bile plugs	Canalicular and ductal/ductular	2 (9)
	Canalicular only	17 (77)
	Ductal/ductular only	0 (0)
	Absent	3 (14)
Pseudorosette formation	Prominent	11 (50)
	Present	9 (41)
	Absent	2 (9)
Lobular mononuclear leukocyte inflammation	Diffuse	5 (23)
	Focal	8 (36)
	Rare	7 (32)
	Absent	2 (9)
Portal inflammation	Generalized, moderate/marked	4 (18)
	Generalized, mild	8 (36)
	Focal, mild	8 (36)
	Absent	2 (9)
Hepatocellular necrosis	Many	4 (18)
	Few	4 (18)
	Absent/rare	14 (64)
Giant cell transformation	Prominent	5 (23)
	Common	1 (5)
	Present	10 (45)
	Rare	6 (27)
Hepatocellular swelling	Prominent	9 (41)
	Present	4 (18)
	Rare	6 (27)
	Absent	3 (14)

TABLE 4

Immunohistochemical Findings and Genetic Abnormalities

Patient	BSEP	Mutation	Type of Mutation(s)
1	Absent	c.890A>G (p.E297G) *	Missense[5,7,10,13,16,19,20]
2	Absent	c.1723C>T (p.R575X)	Nonsense[7,19,20]
		c.2178+1G>T	Noncoding region[20]
3	Present	c.1708G>A (p.A570T)	Missense[20]
		c.3634G>T (p.V1212F)	Missense, predicted deleterious
4	Absent	c.3164T>C (p.L1055P) *	Missense, predicted deleterious
5	Absent	c.3692G>A (p.R1231Q)	Missense[20]
		c.2296G>A (p.G766R)	Missense[20]
6	Absent	c.2782C>T (p.R928X)	Nonsense[13]
		c.3268C>T (p.R1090X)	Nonsense[5,7,13]
7	Present	c.3347G>A (p.G1116E)	Missense, predicted deleterious
		IVS 23-8 G-A	Noncoding region
8	Absent	IVS 16-8 T>G †	Noncoding region[10]
9	Absent	c.2944G>A (p.G982R)	Missense[5,7,19,20]
		c.2296G>A (p.G766R)	Missense[20]
10	Absent	c.2944G>A (p.G982R)	Missense[5,7,19,20]
		c.2296G>A (p.G766R)	Missense[20]
11	Absent	c.319T>C (p.C107R)	Missense, predicted deleterious
		c.611+4A>G	Noncoding region
12	Absent	c.1723C>T (p.R575X)	Nonsense[7,19,20]
		c.2178+1G>T	Noncoding region[20]

* Homozygous.

† Monoallelic mutation.