NR1H4-related Progressive Familial Intrahepatic Cholestasis 5: Further Evidence for Rapidly Progressive Liver Failure

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ABSTRACT

Pathogenic sequence variants in the nuclear bile acid receptor FXR, encoded by NR1H4, have been reported in a small number of children with low-γ-glutamyl transferase (GGT) cholestasis progressing to liver failure. We describe 3 additional children from 2 unrelated families with cholestasis and liver failure because of pathologic variants in NR1H4. One patient underwent liver transplantation and has had good clinical outcomes in 6 years of follow-up. Although that patient has biochemical evidence of increased bile acid synthetic activity, he has not experienced post-transplant diarrhea or allograft steatosis, as has been reported among other transplanted patients.

Key Words: bile acids, coagulopathy, hyperbilirubinemia, jaundice, pediatric

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An advance in our understanding of intrahepatic cholestasis came when the molecular underpinnings of the syndromes known as progressive familial intrahepatic cholestasis (PFIC) type 1, PFIC2, and PFIC3 were found to be because of pathogenic variants in *ATP8B1*, *ABCB11*, and *ABCB4*, respectively. In 2014, pathogenic variants in *TJP2* were linked to low-γ-glutamyl transferase (GGT) cholestasis and termed PFIC4 (1). In 2016, 4 patients in 2 families with low-GGT cholestasis progressing to liver failure were described as having loss-of-function mutations in the nuclear bile acid receptor FXR, encoded by *NR1H4*, a disease subsequently designated PFIC5 (2). Since then, only 1 additional child with PFIC5 has been reported (3). Herein, we describe 3 additional children from 2 unrelated families with cholestasis and liver failure because of pathologic variants in *NR1H4*.

What Is Known

- Pathogenic sequence variants in *NR1H4* are associated with low-γ-glutamyl transferase cholestasis and liver failure.
- Liver transplantation may offer effective rescue of liver failure, but transplanted patients might have residual biochemical and clinical abnormalities owing to extrahepatic FXR expression.

What Is New

- A strict genotype-phenotype correlation may not exist for this condition.
- Increased bile acid synthesis observed among transplanted patients is not universally associated with evidence of allograft injury.

FAMILY 1, PATIENT 1

A 17-month-old Hispanic boy was brought to medical attention for jaundice and abdominal distention. He was the product of an uncomplicated term pregnancy to a 22-year-old primigravida. The patient was normally developing; there was no family history of liver disease, however, the child's parents were consanguineous.

On examination, the patient appeared well-nourished (weight 79th centile, length 47th centile, weight-for-length 91st centile), jaundiced, and his abdomen was distended, with liver and spleen palpable ∼5 cm below the costal margins. Initial laboratory testing is shown in Table 1. Abdominal ultrasonography demonstrated splenomegaly and chest radiography revealed generalized osteopenia, but no evidence for fractures.

A diagnostic evaluation was unrevealing, so a liver biopsy was performed, which showed micronodular cirrhosis and complete absence of BSEP protein along the canaliculus (Fig. 1), while MDR3 expression was preserved, raising the possibility of *ABCB11*-related PFIC2. *ABCB11* gene sequencing, however, was normal.

The patient was treated with parenteral vitamin K, ursodeoxycholic acid, spironolactone, and lactulose. He was hypoglycemic while feeding orally, so dextrose-containing intravenous fluids were initiated along with nasogastric tube feedings. After 2 weeks, his weight gain was suboptimal and he continued to require intravenous dextrose, so parenteral nutrition was started. He was evaluated and approved for liver transplantation, undergoing deceased donor liver transplant 11 weeks after his initial presentation, at 20 months of age. Laboratory testing just before

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TABLE 1. Summary of clinical and laboratory findings

	Family 1		Family 2			
	Patient 1		Patient 2		Patient 3	
Sex	Male		Female		Male	
Age at onset	16 mo		3 wks		1 wk	
Age at initial evaluation	17 mo		1 mo		29 days	
Age at liver transplant	20 mo		NA		NA	
Age at last evaluation	8 y		Died at 8 mo		Died at 7 mo	
Signs	Jaundice, abdominal distention		Jaundice, FTT, respiratory distress, pleural effusion		Jaundice, FTT, respiratory distress, pleural effusion	
Liver biochemistry	Initial evaluation	Before OLT	Initial evaluation	Before death	Initial evaluation	Before death
Direct/conjugated bilirubin (nl <0.3 mg/dL)	11.3	37	5	8.3	8	8.3
AST (nl <60 U/L)	627	291	71	200	78	193
ALT (nl <45 U/L)	383	302	49	69	51	71
GGT (nl 10–19 U/L)	81	72	NM	NM	NM	NM
AFP (nl 8–468 ng/mL)	9,610	NM	NM	NM	>100,000	NM
Ammonia (nl 22–48 μmol/L)	64	84	NM	NM	93	193
Coagulation parameters						
PT (nl 12.9–16.9 sec)	21.8	26.1	NM	NM	15	NM
INR (nl 0.9–1.1)	1.9	2.4	NM	NM	1.25*	NM
Factor V assay (nl 69–132%)	38	26	NM	NM	NM	NM
Factor VII assay (nl 58–150%)	20	13	NM	NM	NM	NM
Platelets (nl 150–450 x10^3/μL)	103	52	NM	NM	308	190

AFP = alpha-fetoprotein; ALT = alanine aminotransferase; AST = aspartate aminotransferase; FTT = failure to thrive; GGT = γ-glutamyl transferase; OLT = orthotopic liver transplant; PT = prothrombin time; INR = international normalized ratio; NA = not applicable; nl = normal; NM = not measured.
*The values obtained were not representative because of transfusions of blood products.

transplant reflected deterioration (Table 1). His postoperative course included systemic hypertension and development of posterior reversible encephalopathy syndrome, which was treated with a change from tacrolimus to sirolimus, antihypertensives, and an antiepileptic. He had an episode of early acute cellular rejection, which was treated satisfactorily with steroids.

Four years after the patient was transplanted, Gomez-Ospina et al (2) described *NR1H4* sequence variants in patients with low-GGT, intrahepatic cholestasis. As their patients exhibited lack of BSEP staining on liver immunohistochemistry, but no evidence for pathologic *ABCB11* sequence variants, like our patient, we pursued trio whole exome sequencing. A homozygous pathogenic variant in *NR1H4* (c.526C>T, p.R176X) was found in the proband and confirmed to be in trans-configuration. Immunostain on retained explanted liver showed complete absence of FXR expression in hepatocytes (Fig. 1).

Six years' posttransplant, the patient has continued to do well clinically. He has not experienced diarrhea and somatic growth is normal. Liver indices have been stably normal on sirolimus monotherapy. Four liver biopsies in the first 2 posttransplant years showed no significant steatosis. Assessment of the bile acid synthesis pathway revealed normal total cholesterol (124 mg/dL), elevated 7-alpha-OH-4-cholesten-3-one (C4) (0.81 μmol/L, normal [nl] 0.02–0.05 μmol/L), and chenodeoxycholic acid (0.78 μmol/L, nl 0.07–0.23 μmol/L), but normal cholic acid (0.11 μmol/L, nl 0.03–0.25 μmol/L).

FAMILY 2, PATIENT 2

A 1-month-old girl of Persian descent presented with jaundice, poor growth, and respiratory distress associated with a pleural effusion. She was the full-term product of a consanguineous (first cousins) union. Initial laboratory testing is shown in Table 1. Diagnostic tests showed normal thyroid-stimulating hormone, 17-OH-progesterone, galactose-1 phosphate uridyl transferase, succinylacetone, and biotinidase. There was no biochemical evidence for disorders of β-oxidation of fatty acids, carnitine metabolism, organic acidemia, or urea cycle defects. Plasma amino acid profile showed elevations of tyrosine, methionine, and galactose interpreted as reflective of hepatic dysfunction.

She experienced a progressive course with increasing jaundice, hypoglycemia, and hyperammonemia (Table 1). She died at 8 months of age because of multiorgan failure.

FAMILY 2, PATIENT 3

Two-and-a-half years later, a 1-week-old boy of the same union was admitted with jaundice, poor growth, and respiratory distress. Like his sister, he was born at term and appeared well at birth. Examination revealed the liver and spleen palpable 2 cm below the costal margins. Initial laboratory testing is shown in Table 1. Ultrasound of the right upper quadrant was normal; however, ultrasound of the chest showed a large, right, pleural effusion. Diagnostic evaluation revealed elevated plasma tyrosine. No succinylacetone was detected, however, and genetic testing for tyrosinemia was also normal. The patient was treated supportively with autologous serum therapy, fresh frozen plasma, and supplemental fat-soluble vitamins but experienced progressive deterioration (Table 1). Whole exome sequencing was performed, which demonstrated a homozygous, out-of-frame insertion, in *NR1H4* (c.276dupT, p.P93Sfs∗4). He died at 7 months of age because of liver failure.

DISCUSSION

We have described 3 patients from 2 unrelated families with *NR1H4* disease. In contrast to most reported patients, Patient 1 had a later onset of illness (16 months) and failure-to-thrive was not a

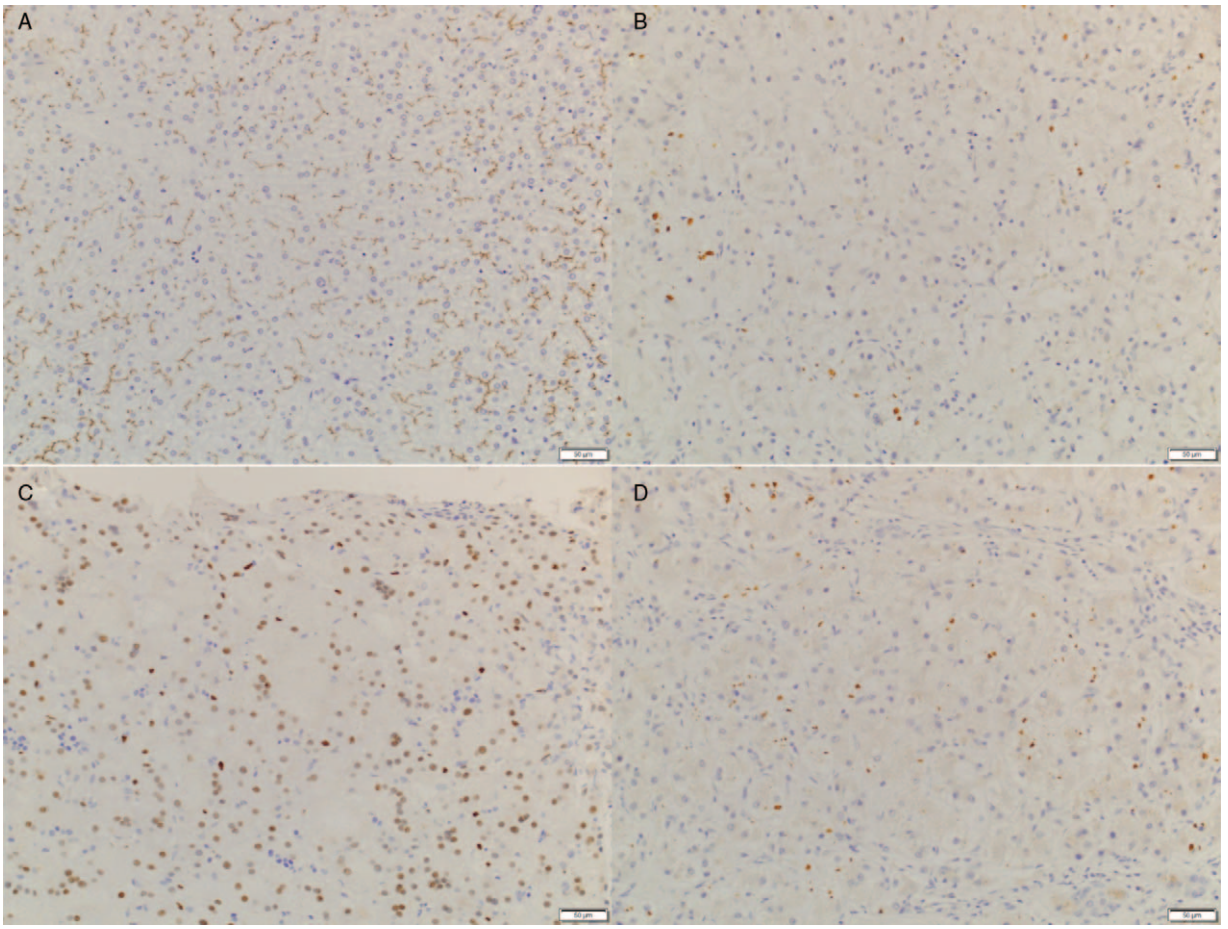

FIGURE 1. Liver immunostain. BSEP normal control (A), and explant of patient 1 (B). FXR normal control (C) and explant of patient 1 (D). BSEP = bile salt excretory protein.

presenting sign. However, like other patients, his course was marked by progressive jaundice, low GGT, vitamin K-refractory coagulopathy, and absence of BSEP staining on liver tissue. Patient 1 has the same homozygous *NR1H4* variant reported in Family 1 from the Gomez-Ospina article, in which both patients had much earlier onset, so a strict genotype-phenotype correlation may not exist. Patient 1 also underwent liver transplantation but has not developed allograft steatosis or liver test abnormalities, in spite of having elevated plasma bile acid precursor C4 posttransplantation, like the transplanted patients in the literature. The increase in bile acid synthesis in these allografts likely relates to loss of FGF19-mediated feedback inhibition on hepatic CYP7A1 stemming from residual loss of the *NR1H4* gene product (FXR) in the native intestine. Patient 1 provides additional evidence that extrahepatic (e.g. gut, kidney) NR1H4 may not be essential as he has not developed a clinical abnormality in either organ system more than 6 years post-transplant.

Regarding Family 2, patients 2 and 3 presented with strikingly similar clinical features and both succumbed to progressive liver disease before the first birthday. Although less clinical and laboratory data was available for these patients, what is reported is consistent with other patients in the literature. The nature of the pleural effusions in these patients, as well as those in the literature (2), is not entirely clear, but that it was documented on the right side in patient 3, may suggest hepatic hydrothorax, a common finding among patients with cirrhosis. Patient 2 died before definitive molecular diagnostics could be obtained; however, whole exome sequencing of patient 3 demonstrated a novel, homozygous, out-of-frame insertion in *NR1H4* (c.276dupT, p.P93Sfs∗4), which is predicted to lead to premature termination of protein translation.

NR1H4-related PFIC5 is a rapidly progressive disease, the natural history of which is not modified by medical interventions. Liver transplantation, on the other hand, appears to provide a satisfactory outcome, with reasonably long follow-up among the small number of patients who have been transplanted. Although this is reassuring, it seems prudent to counsel families of children with PFIC5 being considered for liver transplantation about the extrahepatic expression of NR1H4 as well as the unknowns of gut-liver signaling following transplantation. On the basis of broad differential diagnosis for these patients and limited time in which to make important therapeutic decisions, we would advocate for early use of *NR1H4* sequencing, or unbiased molecular diagnostics like whole exome sequencing, provided results can be available to the clinical team promptly.

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