Expanding the spectrum of progressive familial intrahepatic cholestasis

A report of 3 cases

Jingjing Jiao, MD, PhD,[1] *Raffaella Morotti, MD,*[1] *Nafis Shafizadeh, MD,*[2] *and Dhanpat Jain, MD*[1]

[1]Department of Pathology, Yale School of Medicine, New Haven, CT, US; [2]Department of Pathology, Southern California Permanente Medical Group, Woodland Hills Medical Center, Los Angeles, CA, US

ABSTRACT

Objectives: Progressive familial intrahepatic cholestasis (PFIC) is a group of autosomal recessive disorders caused by defects in bile secretion or transport usually presenting as cholestasis in pediatric age. Herewith, we describe 3 PFIC cases with diagnostic challenges and highlight the role of genetic analysis.

Methods: The clinical history, laboratory data, liver biopsy, and molecular analysis for each case were reviewed.

Results: Case 1, a Hispanic male from Puerto Rico with hepatomegaly since age 2 months, was eventually diagnosed with PFIC3 following identification of a homozygous splice site variant in ATP binding cassette subfamily B member 4 (*ABCB4*) (c.2784-12T>C) at age 17 years by whole-exome sequencing (WES). Case 2 was a 37-year-old man with a history of alcoholism, abnormal liver function tests, and ductopenia on biopsy. Molecular testing revealed a pathogenic heterozygous *ABCB4* mutation (c.1633C>T) variant leading to a diagnosis of PFIC3. Case 3 was a 2-year-old female initially presenting as a drug-induced liver injury but was diagnosed with PFIC10 following identification of a heterozygous frameshift mutation (p.Asp300Trpfs*19) and a heterozygous missense mutation (c.1357T>C) in myosin VB (*MYO5B*) by WES.

Conclusions: These PFIC cases highlight the heterogenous presentation and diagnostic challenges, and they emphasize the role of next-generation sequencing, particularly the utility of WES.

INTRODUCTION

Progressive familial intrahepatic cholestasis (PFIC) is a group of rare, autosomal recessive disorders caused by defects in bile secretion or transport with an estimated incidence of around 1 per 50,000 to 1 per 100,000 births. The main clinical presentations include cholestasis, pruritus, and jaundice in infancy and childhood but rarely in adults as well. Progression is variable, with most patients developing fibrosis and progression to end-stage liver disease before adulthood. Literature review shows that among infants and children with cholestasis, 12% to 13% have genetically diagnosed PFIC.[1] The initial PFIC subtypes identified were PFIC1, PFIC2, and PFIC3, caused by mutations in ATPase phospholipid transporting 8B1 (*ATP8B1*), ATP binding

KEY POINTS

- Progressive familial intrahepatic cholestasis (PFIC) is a group of rare inherited cholestatic disorders with a wide spectrum of clinical presentation.
- Liver biopsy remains critical for the evaluation of PFIC. However, it is important to note that several histologic features can be nonspecific.
- Molecular analysis, particularly whole-exome sequencing, plays a key role in the diagnosis of challenging PFIC cases with atypical clinical presentation and histologic features.

KEY WORDS

γ-glutamyl transferase; whole-exome sequencing; liver fibrosis; *myosin VB* (*MYO5B*); *ATP binding cassette subfamily B member 4* (*ABCB4*); progressive familial intrahepatic cholestasis (PFIC)

Am J Clin Pathol March 2025;163:332-339
HTTPS://DOI.ORG/10.1093/AJCP/AQAE123

Received: June 8, 2024
Accepted: August 22, 2024

Corresponding author: Jingjing Jiao; Jingjing.Jiao@yale.edu

cassette subfamily B member 11 (*ABCB11*), and ATP binding cassette subfamily B member 4 (*ABCB4*), respectively.[2] *ABCB4* encodes a class III multidrug resistance (MDR3) P-glycoprotein, which shows a canalicular pattern of staining by immunohistochemistry in normal liver.[3] With advances in genomic analysis and use of next-generation sequencing (NGS), a growing number of genes have been identified associated with PFIC, and now at least 12 subtypes are recognized.[4,5] Histopathologic features vary among subtypes and include a combination of hepatocellular and canalicular cholestasis, pseud-acini formation, giant cell transformation of hepatocytes, ductular reaction, duct loss, and variable degree of fibrosis. In typical cases, the histologic changes also tend to be characteristic. However, the histologic findings are not specific to any PFIC subtype, and significant heterogeneity exists even within a given subtype. When the disease presents in older patients/adults, often the changes tend to be either milder or atypical, or presentation is with advanced fibrosis/cirrhosis. Here we present 3 unusual cases of PFIC that were diagnosed with the help of molecular testing. In all these cases, the clinical features and the liver histology were nonspecific. These cases not only highlight the wide spectrum of these disorders and challenges in clinical diagnosis but also emphasize the critical role of molecular testing for the diagnosis.

CASE REPORTS

Case 1

The patient was a Hispanic male originally from Puerto Rico with hepatomegaly since 2 months of age with a putative clinical diagnosis of congenital hepatic fibrosis. Laboratory tests showed elevated aspartate transaminase (AST) (74 U/L; normal range, 0-31 U/L) and alanine transaminase (ALT) (124 U/L; normal range, 0-31 U/L), normal alkaline phosphatase (ALK) (307 U/L; normal range, 117-390 U/L), and normal total bilirubin (0.46 mg/dL; normal range, <1.20 mg/dL). Neither bile acid nor γ-glutamyl transferase (GGT) were measured. Liver biopsy at 8 months FIGURE 1A, B demonstrated moderate portal and bridging fibrosis, moderate hepatocellular damage, and mild to moderate degree of bile duct proliferation with no evidence of cholestasis or bile plugging. However, the features were also not typical of ductal plate malformation, and the diagnosis remained unclear. He subsequently developed gallstones, which increased in size and number, leading to cholecystectomy at the age of 10 years. He was also found to have portal hypertension with splenomegaly, left splenorenal shunt, and esophageal varices during surveillance. At age 17 years, he presented with pruritus and direct hyperbilirubinemia (total bilirubin: 2.2 mg/dL [normal range, <1.20 mg/dL]; direct bilirubin: 1.2 mg/dL [normal range, <0.30 mg/dL]) with elevated GGT (373 U/L; normal range, <48 U/L). His serum bile acid level was also elevated at this time (39.8 µmol/L; normal range, ≤6.8 µmol/L). Another liver biopsy was performed and showed cirrhosis FIGURE 1C with moderate portal inflammation FIGURE 1D along with features of chronic cholestasis and ductular proliferation FIGURE 1E. Ceruloplasmin and α-1-antitrypsin levels were normal, and hepatitis B surface antigen and hepatitis C virus antibodies were negative. The diagnosis was unclear, and mutational analysis was performed on peripheral blood for germline mutations. Whole-exome sequencing (WES) identified homozygous splice site mutations in ABCB4 (c.2784-12T>C), supportive of a diagnosis of PFIC3 as the cause of the infantile-onset liver disease. Immunohistochemistry for multiple drug-resistant 3 (MDR3) was subsequently performed; however, it demonstrated normal canalicular expression. FIGURE 1F. Subsequently, the patient has been listed for liver transplantation.

Case 2

A 37-year-old man, who had recently moved to California, presented to his primary care physician stating that he had a "history of liver disease." He was found to have abnormal liver function test about 5 to 6 years ago, when he drank heavily (half a bottle of whiskey per day) and then stopped. He was subsequently referred to the hepatology service and underwent a liver biopsy, which led to a diagnosis of "anti-mitochondrial antibody (AMA)-negative primary biliary cholangitis (PBC)" (slides not available for review). He reported that he was on ursodiol intermittently (last dose 1 year ago) with little improvement in alkaline phosphatase, which remained in the 300 to 500 U/L range. He reported that his mother was also told that she had "AMA-negative PBC."

His current laboratory tests showed ALT of 256 U/L (normal range, 0-63 U/L), AST of 126 U/L (normal range, 0-34 U/L), ALK of 514 U/L (normal range, 0-125 U/L), total bilirubin of 1.3 mg/dL (normal range, <1.0 mg/dL), and GGT of more than 1,200 U/L (greater than the upper limit of the reference range). Anti-nuclear antibody (ANA), AMA, and anti-smooth muscle antibody (ASMA) were all negative. IgG, IgM, and IgA were all within normal limits. Hepatitis C virus antibody and hepatitis B surface antigen (HBsAg) were both negative. The α-1 antitrypsin phenotype was normal (proteinase inhibitor [PI]* MM). A liver biopsy was performed and showed marked ductopenia (5/41 [12%] of portal tracts with bile ducts), with most portal tracts showing minimal to absent lymphocytic inflammation FIGURE 2A, B. There was no significant ductular proliferation, interface activity, steatosis, hepatocyte ballooning, or lobular inflammation. Trichrome stain revealed mild portal fibrous expansion. CK7 immunohistochemistry stain revealed marked chronic cholestatic effect ("biliary metaplasia" of hepatocytes) and confirmed bile duct loss FIGURE 2C. Rhodanine copper stain revealed periportal hepatocyte copper deposition around many of the portal tracts, supporting the histologic impression of chronic cholestasis FIGURE 2D. At this time, the possibility of a germline mutation in a gene encoding for a bile canalicular transport protein, particularly the *ABCB4* gene (coding for the MDR3 protein), was considered. Next-generation sequencing for a cholestatic liver gene panel was performed and revealed a pathogenic heterozygous *ABCB4* mutation (c.1633C>T, p.Arg545Cys), supporting a diagnosis of MDR3 dysfunction (PFIC3) as the cause of the biliary injury. In addition, the same mutation was found in the patient's mother.

Case 3

A 2-year-old previously healthy female presented with cholestasis, pruritus, and jaundice that began after treatment with amoxicillin/

HTTPS://DOI.ORG/10.1093/AJCP/AQAE123

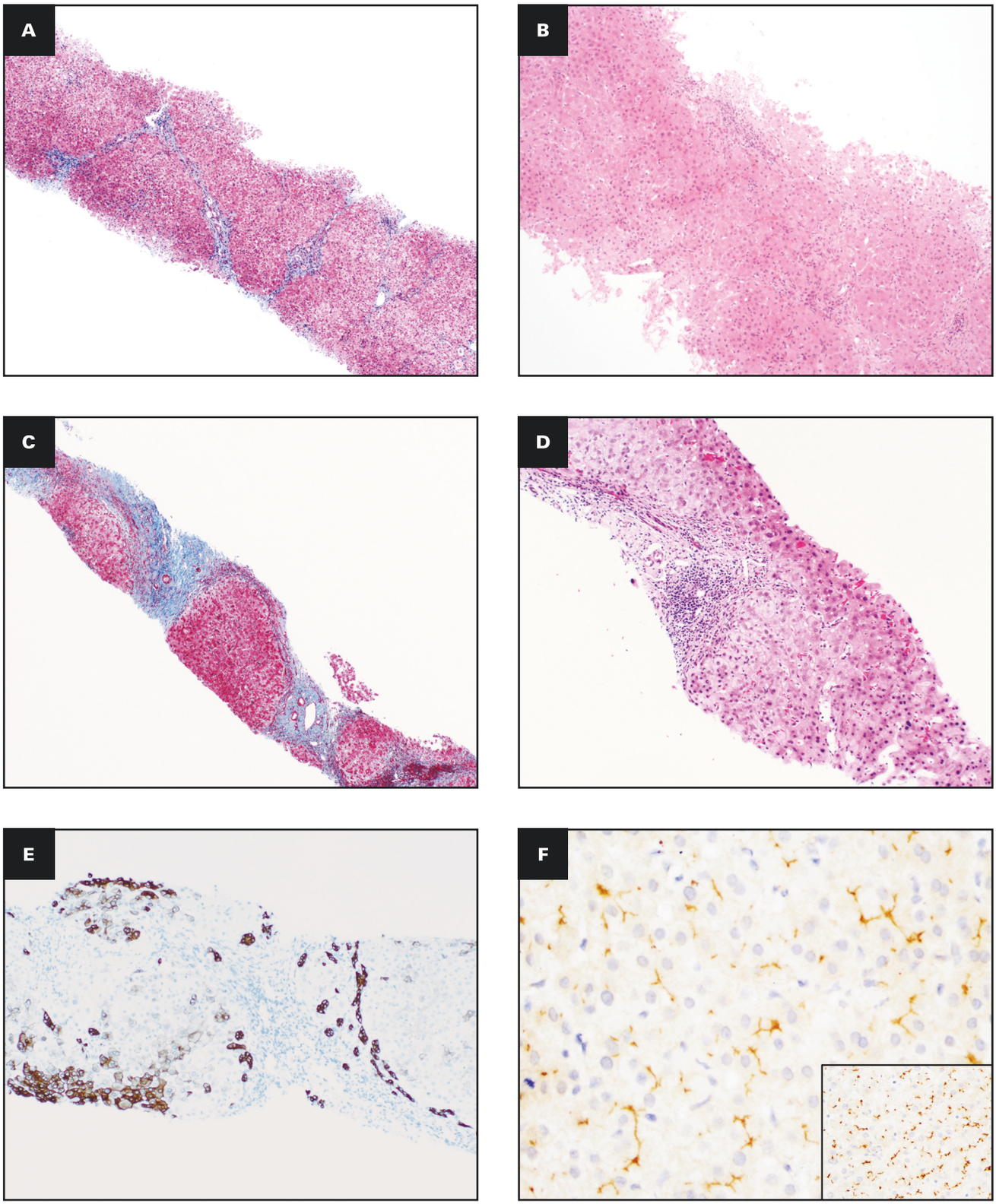

FIGURE 1 Histologic features of case 1. **A**, **B**, Trichome stain (**A**, 4×) and H&E (**B**, 10×) of liver biopsy specimen at 8 months show fibrosis and mild to moderate portal inflammation. **C-E**, Progression to cirrhosis at age 17 years shown by trichrome (**C,** 4×) with moderate portal inflammation (**D**, 10×) and ductular reaction and chronic cholestasis demonstrated by CK7 immunohistochemistry (**E**, 10×). **F**, MDR3 immunohistochemistry compared with positive control (insert) (40×).

HTTPS://DOI.ORG/10.1093/AJCP/AQAE123

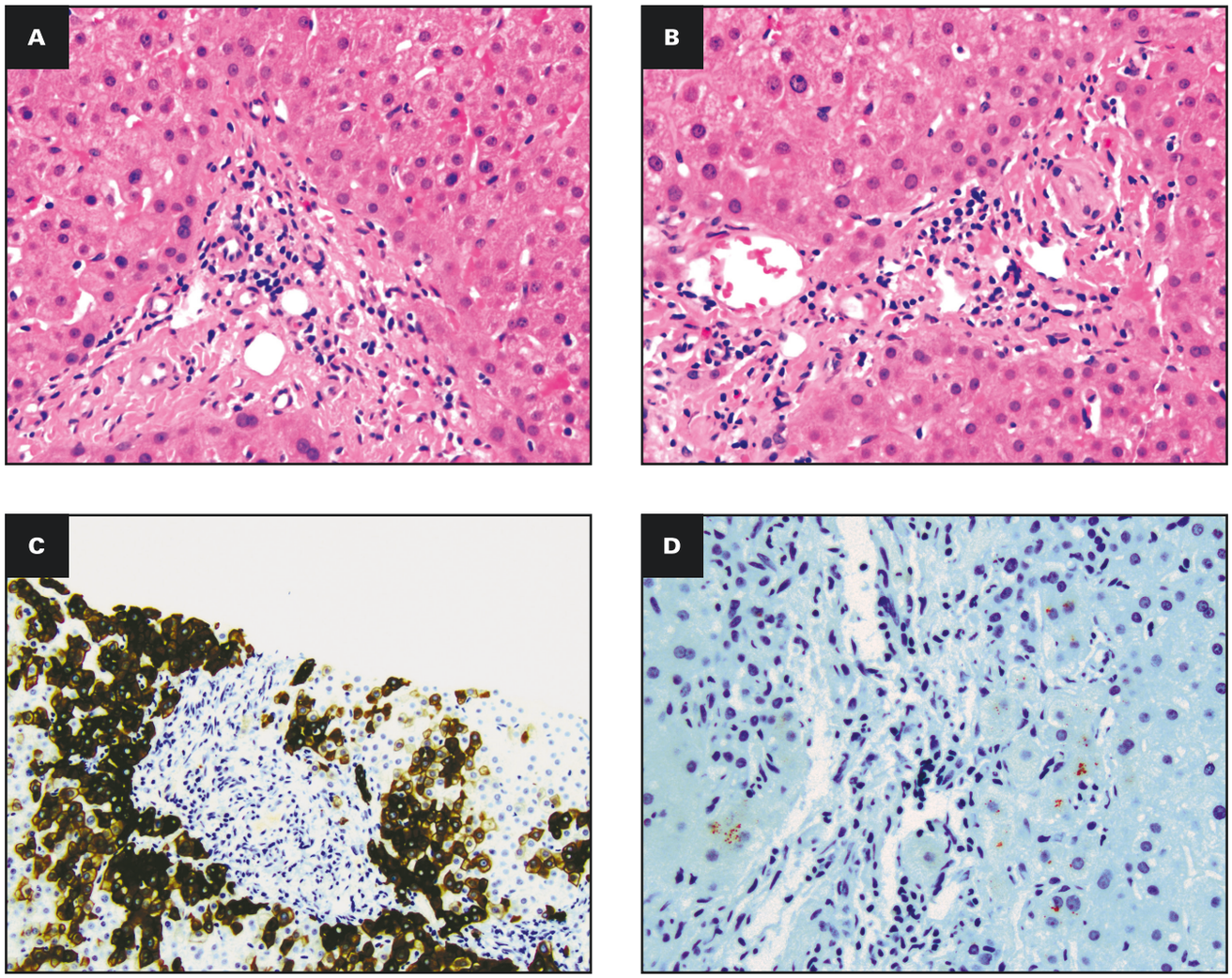

FIGURE 2 Histologic features of case 2. **A**, **B**, Loss of interlobular bile ducts in portal tracts with minimal to absent lymphocytic inflammation and no significant ductular proliferation shown by H&E (40×). **C**, Marked CK7 positivity in periportal hepatocytes ("biliary metaplasia") and the absence of an interlobular bile duct demonstrated by CK7 immunohistochemistry (40×). **D**, Chronic cholestasis shown by rhodanine copper stain (40×).

clavulanate for 5 days for an unclear indication at an outside institution. No diarrhea or abdominal pain was reported. Her family history was unremarkable. The liver function tests showed elevated total and direct bilirubin (total bilirubin: 6.9 mg/dL [normal range, <1.20 mg/dL]; direct bilirubin: 4.9 mg/dL [normal range, <0.20 mg/dL]), ALK (617 U/L; normal range, 117-390 U/L), AST (190 U/L; normal range, 0-31 U/L), and ALT (268 U/L; normal range, 0-31 U/L), and near-normal GGT (33 U/L; normal range, 7-32 U/L). Serum bile acid was also significantly elevated above the clinical reportable range (282 µmol/L; reference range, 4.5-19.2 µmol/L) with elevated chenodeoxycholic acid (80.1 µmol/L; reference range, 9.9 µmol/L or less) and cholic acid, which was also above the clinical reportable range (200 µmol/L; reference range, 3.1 µmol/L or less). Abdominal ultrasound was unremarkable. A liver biopsy was performed and showed paucity of bile ducts with occasional multinucleated giant cells FIGURE 3A-C. A possible drug-induced liver injury was considered, but her symptoms persisted, and bilirubin level remained high months after the cessation of medication. An inherited cholestatic liver disorder including PFIC was then considered, and mutational analysis was performed using a panel for cholestatic disorders at a reference laboratory that did not show mutations in *ATP8B1*, *ABCB11*, *ABCB4*, *JAG1*, or *TJP2*. Subsequently, WES was performed on peripheral blood and found a heterozygous frameshift mutation at codon 300 (c.896_897dupTG; p.Asp300Trpfs*19) and a heterozygous missense mutation (c.1357T>C; p.Phe453Leu) in myosin VB (*MYO5B*). A diagnosis of PFIC10 was made at this time. The patient underwent partial internal biliary drainage surgery with functional end-to-end jejunojejunostomy at age 3 years and remained symptom free with normalization of serum fractionated bile acids at 39 months of follow-up. Subsequently, the patient has been lost to follow-up.

DISCUSSION

Various inherited cholestatic disorders, including PFICs, are rare and can be diagnostically challenging. Delayed diagnosis or misdiagnosis[6,7] is not uncommon, probably due to the wide spectrum of

HTTPS://DOI.ORG/10.1093/AJCP/AQAE123

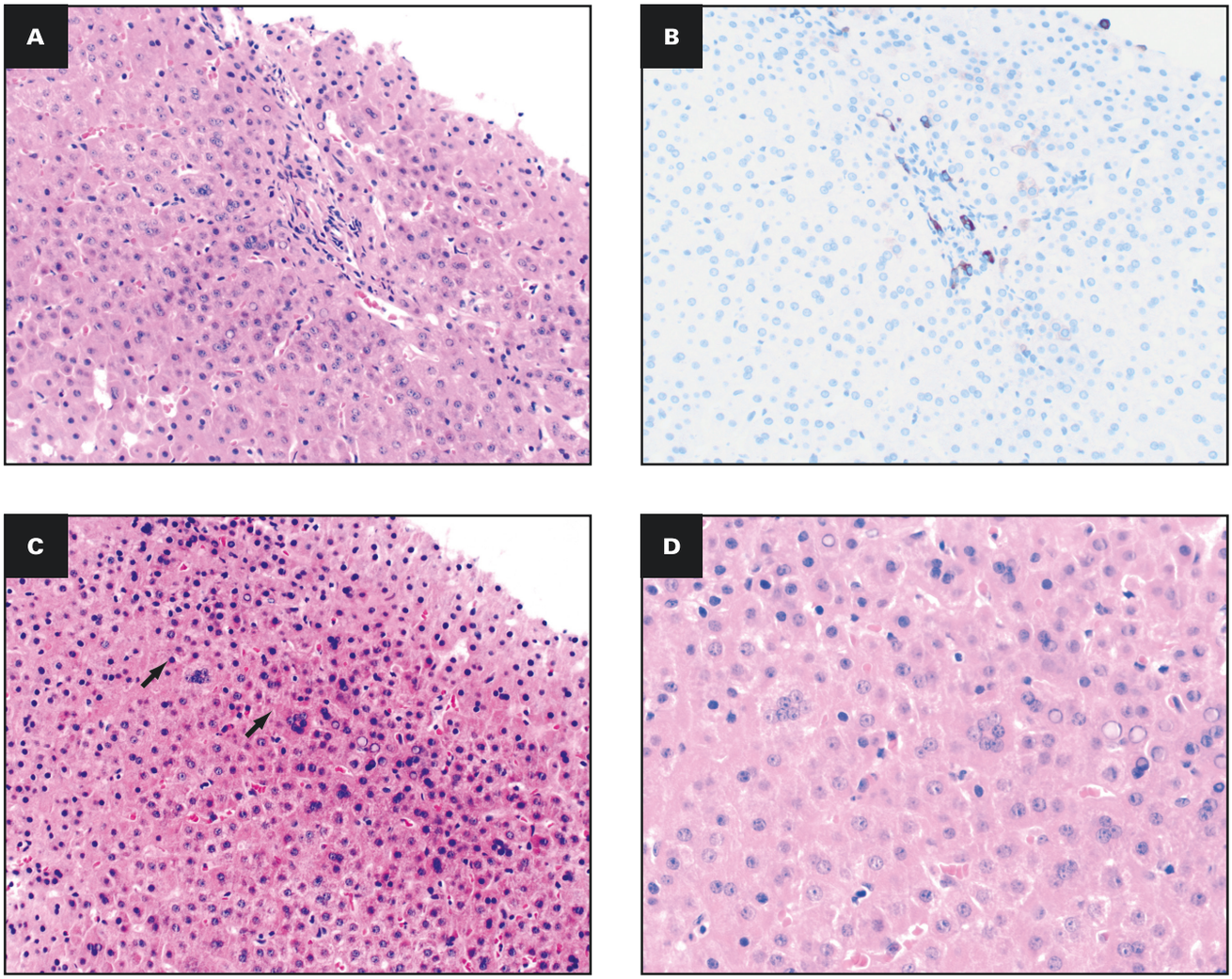

FIGURE 3 Histologic features of case 3. **A**, **B**, Paucity of bile ducts in portal triad shown by H&E (**A**, 20×) and CK7 immunohistochemistry (**B**, 20×). **C**, **D**, Scattered multinucleated giant cells in lobular (arrows) at low power (**C**, 20×) and high power (**D**, 40×).

clinical presentation, lack of awareness among physicians/pathologists, and lack of genetic analysis. However, over the years with advances in genomic medicine and increasing application of NGS in clinical practice, our understanding of these disorders has evolved substantially, and many new PFIC subtypes have been described. It has increasingly become clear that the clinical spectrum of such disorders is very wide, with atypical presentations being not uncommon, and genetic analysis, particularly WES, is very helpful in the diagnostic workup.

In retrospect, the clinical course, laboratory results, and pathology features of case 1 were consistent with PFIC3.[8] However, the diagnosis was not made until the patient developed cirrhosis and molecular studies were pursued. Patients with PFIC3 are at an increased risk of developing cholesterol stones in intrahepatic bile ducts and gallbladder.[9] Histologically, it has 2 patterns at the time of diagnosis: the first pattern shows ductular proliferation with mild inflammation and mild portal fibrosis. Giant cell transformation of hepatocytes and lobular cholestasis can be seen in some cases but remains variable. This can eventually progress to biliary type cirrhosis and liver failure. The second pattern is characterized by extensive portal fibrosis and biliary cirrhosis at presentation.[10] Other features such as subtle bile duct injury[11] and intraductal cholesterol crystals[12] may be observed but are not specific for PFIC3. Complete loss[13] or reduced expression[10,14,15] of MDR3 protein by immunohistochemistry (IHC) can help in the pathologic diagnosis, but retained expression is seen in many cases, indicating a nonfunctional protein, and thus retained MDR3 expression does not exclude the diagnosis of PFIC3.[16,17] The retained MDR3 in case 1 by IHC is explained by the nature of the variant. In case 1, the variant *ABCB4* c.278412T>C occurs in a polypyrimidine tract 12 base pairs (bp) from the 3′ splice site of intron 22 and disrupted splicing, resulting in skipping of exon 23, which leads to a 141-bp deletion and likely encodes for a nonfunctional protein. Normal MDR3 expression by IHC in case 1 suggests retained antigenicity despite loss of function. Clinically, patients with PFIC3 typically show a cholestatic picture with raised liver enzymes, serum bilirubin, and serum bile

HTTPS://DOI.ORG/10.1093/AJCP/AQAE123

salts. The GGT levels are typically high in PFIC3 in contrast with most other PFICs. Presence of extensive portal fibrosis, along with cholestasis and ductular proliferation, helps in establishing the diagnosis on histology in most cases, but none of the findings are entirely specific. Nowadays, a definitive diagnosis is often established by genetic analysis, as happened in this case. The unusual clinical features present in this case included early onset (hepatomegaly at 2 months of age) and lack of jaundice/cholestasis at initial presentation, which led to the consideration of congenital hepatic fibrosis. In addition, the liver biopsy changes were nonspecific, and NGS was not readily available for clinical diagnosis at that time.

The genetic alteration detected in case 1 was also interesting. The variant identified in this case (*ABCB4* c.278412T>C) occurred at the splice acceptor site of intron 22 with a decreased splicing efficiency and was interpreted as likely pathogenic. Interestingly, population-level screening for this variant revealed a carrier rate of 1.95% in Puerto Rican individuals, and incidentally, this patient was also originally from Puerto Rico.[18] A total of 5 individuals homozygous for the *ABCB4* c.278412T>C variant have been reported, of whom 4 were found to have cirrhosis and the fifth had liver steatosis on imaging. Two of these cirrhotic patients underwent liver transplantation, and one was found to have a hepatocellular carcinoma in the explant. Heterozygotes for this variant seem to have associated liver disease in an allele dose-dependent manner.[18] Notably, *ABCB4* mutations have also been associated with increased risk of liver cancer (cholangiocarcinoma and hepatocellular carcinoma), in both case reports[12] and a population-based genomic study.[19]

In case 2, the diagnosis was challenging due to older age at presentation and a liver biopsy specimen that was interpreted as AMA-negative PBC. Eventually, the familial nature of the disease and molecular testing led to the correct diagnosis of PFIC3. The *ABCB4* missense mutation detected in this case, p.Arg545Cys, has been reported in the heterozygous state in multiple individuals with cholestatic liver disease and has also been reported to segregate with disease in at least 1 family with cholestatic phenotype.[20,21] In vitro expression studies have shown that this mutation results in reduced expression of the MDR3 protein, thereby disrupting intracellular transport activity of bile acids.[22] Other variants at this position in the gene (p.Arg545His, p.Arg545Gly) have also been associated with intrahepatic cholestasis, suggesting that a change at this position adversely affects protein structure and/or function.[14,23] The patient's mother was also found to have the same mutation, confirming that the disease was inherited. The other interesting aspect is that his mutation is heterozygous and missense, which presents with less severe or even nonprogressive forms of MDR3 dysfunction, as opposed to large deletions, nonsense mutations, or frameshift mutations that are more common in the severe and pediatric forms of the disease. The histologic presentation of ductopenia is also noteworthy; as discussed earlier, cholestasis with ductular proliferation is the more common histologic pattern of injury in PFIC3. This case suggests that unexplained ductopenia should always raise the possibility of an *ABCB4* mutation, even in adults, particularly in cases with high GGT levels.

Case 3 presented as a low-GGT cholestasis with jaundice and pruritus without significant gastrointestinal manifestation associated with 2 heterogenous variants in *MYO5B*. Isolated low-GGT cholestasis due to a variant in actin filament–based motor protein myosin VB was first reported by 2 groups independently in 2017.[24] It was initially designated as PFIC6 in literature,[25] but subsequently, it has been recognized in the Online Mendelian Inheritance in Man as PFIC10, phenotype 619658. The protein myosin VB binds selected small guanosine triphosphatase rab proteins, including the trans-Golgi network–associated and/or recycling endosome-associated rab8 and rab11a, and has been implicated in regulating membrane trafficking in polarized epithelial cells such as hepatocytes, enterocytes, and respiratory epithelial cells.[25] Biallelic *MYO5B* mutations have been identified in patients with a spectrum of clinical manifestations, ranging from microvillus inclusion disease (MYO5B-MVID), a congenital enteropathy characterized by intractable diarrhea and malabsorption and, at the cellular level, the mislocalization of apical brush-border proteins, an enteropathy associated with cholestatic liver disease (MYO5B-MIXED)[26] or a cholestatic liver disease (MYO5B-PFIC, PFIC10).[27] Clinically, patients with typical PFIC10 have low GGT, raised bilirubin, and/or bile acid similar to other PFICs, such as PFIC1 and PFIC2. Neurologic symptoms such as delayed language development and a pyramidal syndrome have been reported in 1 case. Histologic features include intralobular cholestasis, giant cell transformation of hepatocytes, and a variable degree of fibrosis.[24,28] Reduced or abnormal bile salt export pump expression is seen in a subset of patients.[24,27] Our patient presented with similar characteristics to those previously described, with normal GGT, PFIC-like phenotype, and a disease onset in the first 2 years of life. It is possible that the clinical presentation was triggered or worsened by antibiotics (amoxicillin/clavulanate), which has not been described before. Lack of specific features and familiarity with rare diseases like this often leads to delayed or misdiagnosis, and genetic evaluation remains a key similar to this case.

In summary, we here present 3 cases of PFIC disorders that highlight the challenges in clinical diagnosis and emphasize the key role of genetic analysis, particularly the utility of WES. Key features of each case are summarized in **TABLE 1**. Liver biopsy remains critical for the evaluation of pediatric cholestatic disorders, including PFICs, and generates a good differential diagnosis to guide appropriate genetic workup. As the PFIC classification continues to expand, it is equally important to note that several histologic features are common to different molecular subtypes of PFICs (eg, giant cell transformation of hepatocytes, hepatocellular and canalicular cholestasis, ductular proliferation), and pathologists need to be aware of this. Molecular testing has evolved from direct sequencing of a single gene to panel-based NGS to WES and has become an essential component of the workup.[29] The problems encountered with genetic analysis using standard or preset panels are that new variants or alterations in genes excluded from the panel can be missed, as happened with one of the cases presented here, while whole-exome/genome analysis tends to frequently detect new variants, including variants of unknown significance, making the interpretation challenging. However, whole-exome and genome sequencing are becoming increasingly available and increasingly used in research and clinical practice, and subsequently, expertise in

HTTPS://DOI.ORG/10.1093/AJCP/AQAE123

TABLE 1 Key Features of Each Case of Progressive Familial Intrahepatic Cholestasis

Case No.	Age, y/sex	Age at presentation	Presenting symptoms	Key laboratory values	Key histologic features	Genetics
1	17 y, male	8 mo	Hepatomegaly for 6 mo	AST: 74 U/L ALT: 124 U/L ALK: 307 U/L Total bilirubin: 0.46 mg/dL	Moderate portal and bridging fibrosis, moderate hepatocellular damage and mild to moderate degree of bile duct proliferation with no evidence of cholestasis or bile plugging	*ABCB4* homozygous variants (c.2784-12T>C)
2	37 y, male	31 y	Abnormal liver function tests	AST: 126 U/L ALT: 256 U/L ALK: 514 U/L Total bilirubin: 1.3 mg/dL GGT: >1200 U/L	Marked paucity of bile ducts in the absence of significant ductular proliferation	*ABCB4* heterozygous mutation (c.1633C>T)
3	2 y, female	2 y	Cholestasis, pruritus, and jaundice 5 days after antibiotic treatment	AST: 190 U/L ALT: 268 U/L ALK: 617 U/L Total bilirubin: 6.9 mg/dL Direct bilirubin: 4.9 mg/dL GGT: 33 U/L Bile acid: 282 µmol/L	Paucity of bile ducts with occasional multinucleated giant cells	*MYO5B* heterozygous variants (c.896_897dupTG; p.Asp300Trpfs*19 and c.1357T>C; p.Phe453Leu)

ABCB4, ATP binding cassette subfamily B member 4; ALK, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; GGT, γ-glutamyl transferase; *MYO5B*, myosin VB.

their interpretation is also developing rapidly.[30-32] This has clearly widened our understanding of the clinical spectrum, as well as identified new mutations or genetic variations associated with established disorders of bile synthesis and transport, along with discovery of new disorders. Thus, the list of new subtypes of PFICs has grown over the past decade and continues to evolve. Hence, cases reported like this help guide not only the clinical practice but also future research in this area.

Conflict of interest disclosure: The authors have nothing to disclose.

Funding: None

REFERENCES

1. Baker A, Kerkar N, Todorova L, Kamath BM, Houwen RHJ. Systematic review of progressive familial intrahepatic cholestasis. *Clin Res Hepatol Gastroenterol.* 2019;43(1):20-36. https://doi.org/10.1016/j.clinre.2018.07.010
2. Davit-Spraul A, Gonzales E, Baussan C, Jacquemin E. Progressive familial intrahepatic cholestasis. *Orphanet J Rare Dis.* 2009;4:1. https://doi.org/10.1186/1750-1172-4-1
3. Srivastava A. Progressive familial intrahepatic cholestasis. *J Clin Exp Hepatol.* 2014;4(1):25-36. https://doi.org/10.1016/j.jceh.2013.10.005
4. Hassan S, Hertel P. Overview of progressive familial intrahepatic cholestasis. *Clin Liver Dis.* 2022;26(3):371-390. https://doi.org/10.1016/j.cld.2022.03.003
5. Xie S, Wei S, Ma X, et al. Genetic alterations and molecular mechanisms underlying hereditary intrahepatic cholestasis. *Front Pharmacol.* 2023;14:1173542. https://doi.org/10.3389/fphar.2023.1173542
6. Schatz SB, Jüngst C, Keitel-Anselmo V, et al. Phenotypic spectrum and diagnostic pitfalls of ABCB4 deficiency depending on age of onset. *Hepatol Commun.* 2018;2(5):504-514. https://doi.org/10.1002/hep4.1149
7. Boga S, Jain D, Schilsky ML. Presentation of progressive familial intrahepatic cholestasis type 3 mimicking Wilson disease: molecular genetic diagnosis and response to treatment. *Pediatr Gastroenterol Hepatol Nutr.* 2015;18(3):202-208. https://doi.org/10.5223/pghn.2015.18.3.202
8. Deleuze JF, Jacquemin E, Dubuisson C, et al. Defect of multidrug-resistance 3 gene expression in a subtype of progressive familial intrahepatic cholestasis. *Hepatology.* 1996;23(4):904-908. https://doi.org/10.1002/hep.510230435
9. Davit-Spraul A, Gonzales E, Baussan C, Jacquemin E. The spectrum of liver diseases related to ABCB4 gene mutations: pathophysiology and clinical aspects. *Semin Liver Dis.* 2010;30(2):134-146. https://doi.org/10.1055/s-0030-1253223
10. Jacquemin E, De Vree JM, Cresteil D, et al. The wide spectrum of multidrug resistance 3 deficiency: from neonatal cholestasis to cirrhosis of adulthood. *Gastroenterology.* 2001;120(6):1448-1458. https://doi.org/10.1053/gast.2001.23984
11. Stattermayer AF, Halilbasic E, Wrba F, Ferenci P, Trauner M. Variants in ABCB4 (MDR3) across the spectrum of cholestatic liver diseases in adults. *J Hepatol.* 2020;73(3):651-663. https://doi.org/10.1016/j.jhep.2020.04.036
12. Wendum D, Barbu V, Rosmorduc O, Arrivé L, Fléjou J-F, Poupon R. Aspects of liver pathology in adult patients with MDR3/ABCB4 gene mutations. *Virchows Arch.* 2012;460(3):291-298. https://doi.org/10.1007/s00428-012-1202-6
13. de Vree JM, Jacquemin E, Sturm E, et al. Mutations in the MDR3 gene cause progressive familial intrahepatic cholestasis. *Proc Natl Acad Sci U S A.* 1998;95(1):282-287. https://doi.org/10.1073/pnas.95.1.282
14. Wang Z, Yang F, Wang J, Ma X. A novel mutation of ABCB4 in progressive familial intrahepatic cholestasis 3: like mother, like daughter. *J Clin Gastroenterol.* 2016;50(4):353-354. https://doi.org/10.1097/MCG.0000000000000474
15. Frider B, Castillo A, Gordo-Gilart R, et al. Reversal of advanced fibrosis after long-term ursodeoxycholic acid therapy in a patient with residual expression of MDR3. *Ann Hepatol.* 2015;14(5):745-751.
16. Dzagania T, Engelmann G, Häussinger D, et al. The histidine-loop is essential for transport activity of human MDR3. A novel mutation of MDR3 in a patient with progressive familial intrahepatic cholestasis type 3. *Gene.* 2012;506(1):141-145. https://doi.org/10.1016/j.gene.2012.06.029
17. Keitel V, Burdelski M, Warskulat U, et al. Expression and localization of hepatobiliary transport proteins in progressive familial intrahepatic cholestasis. *Hepatology.* 2005;41(5):1160-1172. https://doi.org/10.1002/hep.20682
18. Belbin GM, Rutledge S, Dodatko T, et al. Leveraging health systems data to characterize a large effect variant conferring risk for liver disease in Puerto Ricans. *Am J Hum Genet.* 2021;108(11):2099-2111. https://doi.org/10.1016/j.ajhg.2021.09.016

19. Gudbjartsson DF, Helgason H, Gudjonsson SA, et al. Large-scale whole-genome sequencing of the Icelandic population. *Nat Genet.* 2015;47(5):435-444. https://doi.org/10.1038/ng.3247
20. Degiorgio D, Crosignani A, Colombo C, et al. ABCB4 mutations in adult patients with cholestatic liver disease: impact and phenotypic expression. *J Gastroenterol.* 2016;51(3):271-280. https://doi.org/10.1007/s00535-015-1110-z
21. Pauli-Magnus C, Kerb R, Fattinger K, et al. BSEP and MDR3 haplotype structure in healthy Caucasians, primary biliary cirrhosis and primary sclerosing cholangitis. *Hepatology.* 2004;39(3):779-791. https://doi.org/10.1002/hep.20159
22. Andress EJ, Nicolaou M, McGeoghan F, Linton KJ. ABCB4 missense mutations D243A, K435T, G535D, I490T, R545C, and S978P significantly impair the lipid floppase and likely predispose to secondary pathologies in the human population. *Cell Mol Life Sci.* 2017;74(13):2513-2524. https://doi.org/10.1007/s00018-017-2472-6
23. Ziol M, Barbu V, Rosmorduc O, et al. ABCB4 heterozygous gene mutations associated with fibrosing cholestatic liver disease in adults. *Gastroenterology.* 2008;135(1):131-141. https://doi.org/10.1053/j.gastro.2008.03.044
24. Qiu YL, Gong J-Y, Feng J-Y, et al. Defects in myosin VB are associated with a spectrum of previously undiagnosed low gamma-glutamyltransferase cholestasis. *Hepatology.* 2017;65(5):1655-1669. https://doi.org/10.1002/hep.29020
25. Overeem AW, Li Q, Qiu Y-L, et al. A molecular mechanism underlying genotype-specific intrahepatic cholestasis resulting from MYO5B mutations. *Hepatology.* 2020;72(1):213-229. https://doi.org/10.1002/hep.31002
26. Girard M, Lacaille F, Verkarre V, et al. MYO5B and bile salt export pump contribute to cholestatic liver disorder in microvillous inclusion disease. *Hepatology.* 2014;60(1):301-310. https://doi.org/10.1002/hep.26974
27. Matarazzo L, Bianco AM, Athanasakis E, et al. MYO5B gene mutations: a not negligible cause of intrahepatic cholestasis of infancy with normal gamma-glutamyl transferase phenotype. *J Pediatr Gastroenterol Nutr.* 2022;74(5):e115-e121. https://doi.org/10.1097/MPG.0000000000003399
28. Wang L, Qiu Y-L, Xu H-M, et al. MYO5B-associated diseases: novel liver-related variants and genotype-phenotype correlation. *Liver Int.* 2022;42(2):402-411. https://doi.org/10.1111/liv.15104
29. Chen HL, Li H-Y, Wu J-F, et al. Panel-based next-generation sequencing for the diagnosis of cholestatic genetic liver diseases: clinical utility and challenges. *J Pediatr.* 2019;205:153-159.e6. https://doi.org/10.1016/j.jpeds.2018.09.028
30. Kulecka M, Habior A, Paziewska A, et al. Clinical applicability of whole-exome sequencing exemplified by a study in young adults with the advanced cryptogenic cholestatic liver diseases. *Gastroenterol Res Pract.* 2017;2017:1-8. https://doi.org/10.1155/2017/4761962
31. Hakim A, Zhang X, DeLisle A, et al. Clinical utility of genomic analysis in adults with idiopathic liver disease. *J Hepatol.* 2019;70(6):1214-1221. https://doi.org/10.1016/j.jhep.2019.01.036
32. Vilarinho S, Choi M, Jain D, et al. Individual exome analysis in diagnosis and management of paediatric liver failure of indeterminate aetiology. *J Hepatol.* 2014;61(5):1056-1063. https://doi.org/10.1016/j.jhep.2014.06.038