Overview of Progressive Familial Intrahepatic Cholestasis

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KEYWORDS

• PFIC • Cholestasis • FIC1 • BSEP • MDR3 • TJP2 • MYO5B • FXR • USP53

KEY POINTS

- The progressive familial intrahepatic cholestasis disorders are a heterogeneous group of disorders that result from disruption of bile secretion and lead to cholestasis, pruritus, and/or progressive liver disease.
- Management includes supportive care, including treatment of pruritus, and addressing complications of chronic liver disease. Liver transplant may be required for end-stage liver disease, intractable pruritus, and/or hepatocellular carcinoma.
- Surgical interruption of enterohepatic circulation or novel pharmacotherapy resulting in inhibition of ileal bile acid transporters lead to increased excretion of bile acids from the gut and may improve pruritus.
- Although liver transplantation remains the definitive therapy for medically refractory disease, it does not alleviate extrahepatic manifestations associated with familial intrahepatic cholestasis 1 (FIC1) or tight junction protein 2 disease and carries a risk of disease in the transplanted organ in certain patients with FIC1, bile salt export pump, or farnesoid X receptor disease.

INTRODUCTION

Progressive familial intrahepatic cholestasis (PFIC) is a label applied to a heterogenous group of monogenic disorders that cause impaired intrahepatic bile flow or cholestasis.[1–4] These cholestasis syndromes result from defects in canalicular bile acid trafficking and/or secretion, and comprise a broad clinical spectrum ranging from a nonprogressive, intermittent cholestatic jaundice (benign recurrent intrahepatic cholestasis, or "BRIC"), to chronic liver disease (PFIC).[5,6] Their heterogeneity and rarity have made study of these disorders challenging but collaborative, multicenter

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studies such as the Natural Course and Prognosis of PFIC and Effect of Biliary Diversion (NAPPED) and the Childhood Liver Disease Research Network consortia have enabled study of larger cohorts. The PFIC disorders are autosomal recessive but individuals with heterozygous mutations in these genes may present with transient neonatal cholestasis, intrahepatic cholestasis of pregnancy (ICP), drug-induced liver injury, cholelithiasis/choledocholithiasis, predisposition to parenteral nutrition associated liver disease, or milder forms of chronic multidrug resistance 3 (MDR3) liver disease.[7–12] Homozygous nonsense or frameshift mutations or large deletions, resulting in a nonfunctional protein, may be more likely to result in severe disease than mutations that only partially affect function but clear genotype–phenotype relationships are not always present.[13–15] Pruritus remains one of the most significant clinical manifestations of these disorders, and may severely affect sleep, school performance, and overall quality of life. Severe, refractory pruritus may be an indication for liver transplant. End-stage liver disease and/or liver cancer also necessitate liver transplant. With advances in molecular genetics, additional PFIC disorders have been identified in recent years, further highlighting the burden of genetic causes of intrahepatic cholestasis.[2,5,6] The purpose of this review is to provide a comprehensive outline of genetic causes, clinical manifestations, and management strategies for the evolving class of monogenic intrahepatic cholestasis disorders referred to as PFIC.

DISCUSSION

Bile Flow and Metabolism

Bile, an alkaline fluid, is a robust detergent that is produced by hepatocytes and contains bilirubin, bile acids, cholesterol, and other lipids.[5,16] It is typically transported via the biliary tree and stored in the gallbladder, where it is released into the small intestine to aid in lipid digestion and fat-soluble vitamin absorption.[17] Additionally, it removes toxins from the liver and facilitates their excretion into the gut.[18] Once excreted, some bile acids are reabsorbed in the ileum and transported back to the liver via enterohepatic circulation by the apical sodium-dependent bile transporter (ASBT).[2,3,5]

Bile acid trafficking is a complex physiologic process that depends on intricate machinery to ensure smooth transport out of the hepatocyte. The bile salt export pump (BSEP) is essential in transporting bile salts formed by hepatocytes across the canalicular membrane, the stability of which rests on an adequate enclosure and dispersal of the phosphatidylcholine molecule regulated by MDR3 and familial intrahepatic cholestasis 1 (FIC1) proteins, respectively.[3,5,19] BSEP expression itself is controlled by farnesoid X receptor (FXR), and its localization on the canalicular membrane depends on myosin 5B (MYO5B).[2] The integrity of hepatocytes is partially determined by tight junctions (whose integrity depends on tight junction protein 2 [TJP2]) that protect hepatocytes from the detergent properties of bile and, likely, ubiquitin-specific protease 53 (USP53), which has been shown to colocalize and interact with TJP2 in mice.[20] Genetic defects in any of these proteins may lead to intrahepatic cholestasis.

Epidemiology and Prevalence

Incidence of the PFIC disorders is difficult to precisely determine due to relative infrequency and heterogeneity of this class of disorders, and lack of genetic data to confirm diagnosis in many older studies. As cited in a recent systematic review, the local population prevalence of PFIC was between 9% and 12.9% of children admitted to hospital with cholestasis, liver failure, or splenomegaly based on 3 studies.[21] The first 3 identified PFIC disorders, FIC1 disease, BSEP disease, and MDR3 disease

are the most common, with BSEP disease being the most common out of these 3 diseases.[7,22] For patients with severe FIC1 or BSEP deficiency, survival with native liver (SNL) beyond childhood is not expected in most cases, with only 50% surviving with their native liver up to 10 years and nearly none to 20 years of age in one study.[23] Patients with milder forms of disease can be expected to survive with their native liver well into adulthood.

Pruritus in Progressive Familial Intrahepatic Cholestasis Disorders

Pruritus is a hallmark symptom of the PFIC disorders, and it can be severely debilitating.[22] It is treated with medications and/or nontransplant surgery (surgical interruption of enterohepatic circulation; sEHC) to interrupt enterohepatic circulation of bile acids. In the most severe and refractory cases, it can be an indication for liver transplant. The pathophysiology of pruritus in cholestatic liver disease is not well understood. It is thought that circulating bile acids play a role but serum bile acid levels do not always correlate with pruritus symptoms. There is a more direct correlation between itching and circulating levels of lysophosphatidic acid (LPA), a neuronal activator, and levels of autotaxin, the enzyme responsible for the formation of LPA.[24] The ItchRO scale was recently developed as a tool to objectively assess pruritus, and it has proven useful in assessing response to treatment.[25]

Medications for Pruritus

Table 1 summarizes medications commonly used to manage pruritus in PFIC disorders. Ursodeoxycholic acid, or ursodiol, is a synthetic hydrophilic bile acid used in many cholestatic liver disorders used to alleviate pruritus and improve bile flow. It renders bile more hydrophilic (less toxic to cell membranes) and promotes bile flow by stimulating cholangiocyte bicarbonate secretion and upregulating BSEP and MRP2.[26] It can prevent biliary stone formation (BSEP and MDR3 disease) and, in some cases, it may slow or reverse the progression of disease.[27] Rifampicin (rifampin) may exert antipruritic effects via its enhancement of hepatic cytochrome P450 activity and increased 6-a hydroxylation and 2-a glucuronidation of bile acids and/or by its effects on intestinal flora and secondary bile acids.[28] Other therapies for pruritus include bile acid-binding resins (including cholestyramine), antihistamines (hydroxyzine, diphenhydramine), naltrexone, and sertraline. Therapy using molecular chaperones such as 4-phenylbutyrate, which may rescue protein function associated with missense BSEP and FIC1 mutations in vitro, has promise but caution and further study are warranted after the first participant (with FIC1 deficiency) enrolled in a 4-phenylbutyrate trial developed severe, acute, reversible liver injury after withdrawal of rifampin, which had been prescribed concomitantly. It was hypothesized that discontinuation of the rifampin, a strong inducer of CYP3A4, resulted in phenylacetate toxicity.[29] Ileal apical sodium-dependent intestinal bile acid transporters inhibitors (iBAT inhibitors) are a newer class of antipruritus medications that work by reducing bile reabsorption, which occurs primarily in the ileum, back into enterohepatic circulation. Odevixibat is the first FDA-approved (July 2021) medical therapy for pruritus in patients 3 months of age and older with all types of PFIC.[30] In trials, once-daily administration was shown to reduce serum bile acids and improve pruritus and associated sleep disturbance in children with cholestasis. It may also improve hepatic fibrosis, although the mechanism remains unclear. Drug is primarily confined to the intestinal lumen and is generally well tolerated, with diarrhea, fat-soluble vitamin deficiency, and transient increases in aminotransferases being the most common side effects. No serious adverse events were reported in trials before FDA approval.[30] There are several other iBAT inhibitors currently in development.[22]

Table 1
Pharmacotherapy for alleviating pruritus

Drug Name	Mechanism of Action	Comments
Ursodeoxycholic acid (ursodiol)	Choleretic	Promotes bile flow May improve hepatic fibrosis in some case
Hydroxyzine	Antihistamine	May cause drowsiness
Cholestyramine Colesevelam Colestipol	Bile-acid binding resin/ sequestrant	Gastrointestinal side effects include nausea, vomiting, bloating and constipation May be unpalatable Administer at least 1 h after and 4 h before other medications
Rifampicin, Rifampin	Antibiotic, potent agonist of pregnane-X-receptor, which promotes detoxification	Association with development of hepatotoxicity 10%–15% Contraindicated in patients with advanced liver disease
Naltrexone, Naloxone	Opioid antagonist	Can be associated with opioid withdrawal-like symptoms Variable rates of elevated liver enzymes in some cases. Use of opioid antagonists may complicate control of acute or postoperative pain unless dosong held at least 72 h
Sertraline	Selective serotonin reuptake inhibitor	Mechanism of action in treatment of pruritus is unclear
Odevixibat	Intestinal bile acid transport inhibitor	Diarrhea or other GI discomfort is most common side effect. May cause elevated liver enzymes. Limited efficacy in patients with BSEP deficiency with complete absence of BSEP protein

Nontransplant Surgery for Pruritus

Surgical interventions ("sEHC" hereafter) are available when medical management has been maximized but incompletely effective and include partial biliary diversion (PEBD) and ileal bypass.[22,31] PEBD can decrease serum bile acids by disrupting the enterohepatic circulation via surgical placement of an external biliary conduit.[32,33] Complications may include diarrhea, profuse stoma output with dehydration and/or electrolyte abnormalities, and surgical complications necessitating revisions.[34,35] A trial of nasobiliary drainage, using a nasogastric feeding tube, may be attempted in surgical candidates to assess response before committing to surgery.[36] PEBD should be avoided

in patients with cirrhosis because a beneficial response may be less likely, and varices can develop at the stoma site and cause bleeding if portal hypertension is present. Partial internal biliary diversion is a more recently developed surgical procedure that consists of creating an internal conduit between the gallbladder and the colon and bypassing the terminal ileum, the site of bile acid reabsorption, thereby effectively disrupting the enterohepatic circulation but without necessitating creation of an external ostomy.[35,37] Choleretic diarrhea may result from bile flow directly into the colon, for which bile acid sequestrants may be helpful in management. There is also a theoretic long-term cancer risk associated with bile flowing directly into the colon, which should be considered after internal diversion.[38] Both types of sEHC surgeries may lead to improved laboratory and growth parameters, improved hepatic fibrosis, and better control of pruritus.[15,32,35,39] Ileal exclusion is performed to decrease bile acid reabsorption by surgically bypassing 15% to 20% of the distal ileum, where most bile acid reabsorption occurs. It is favored in patients with gallbladder anomalies or, particularly before the development of internal biliary diversion, in those who wished to avoid or to close an existing PEBD stoma. It can, however, lead to significant diarrhea and malabsorption, and its effectiveness in controlling pruritus is often transient.[40]

Specific Progressive Familial Intrahepatic Cholestasis Syndromes

Here forth, specific PFIC syndromes, listed based on affected protein and gene, are discussed. **Table 2** summarizes several genetic variants that may cause intrahepatic cholestasis and cites associated clinical manifestations of each disorder.

Familial intrahepatic cholestasis 1 (Byler) disease: ATPase phospholipid transporting 8B1 gene (OMIM 211600, 243300)

Originally identified in the family of Jacob Byler in Western Pennsylvania in late 1960s, FIC1 deficiency, also commonly known as "Byler" disease, represents the first recognized type of progressive intrahepatic familial cholestasis. It is also termed PFIC 1.[16]

Familial intrahepatic cholestasis 1 disease genetics and pathophysiology. Mutations in the *ATPase phospholipid transporting 8B1 gene (ATP8B1)* gene on chromosome 8 cause FIC 1 disease, also called PFIC 1 or "Byler" disease. The protein FIC1 is expressed on hepatocytes and contributes to the stability of the canalicular membrane. It is a member of the ATP-dependent membrane transporters; specifically, ATP8B1—a phospholipid flippase, which translocates phospholipids inward on the cell membrane.[6] Both flippases and floppases, the latter of which translocate phospholipids outward on the cell membrane, maintain a balanced distribution of lipids in the cell membrane. It is hypothesized that mutations in FIC1 result in impaired bile flow due to membrane instability and resultant inability to effectively traffic bile acids, which accumulate intracellularly and become cytotoxic. In addition to its hepatic expression, FIC1 is also expressed in the ear, pancreas, small intestine, and bladder, which is relevant to its role in extrahepatic disease and in complications that may develop following liver transplant [12]. A mild disease phenotype is commonly associated with at least one mutation that affects protein function only moderately, including common missense mutation I661 T, which is the most common mutation associated with a BRIC phenotype.[36] Genotype alone does not, however, tend to reliably predict disease course or response to sEHC in FIC1 disease in general.[15,32,39]

Familial intrahepatic cholestasis 1 disease clinical manifestations, laboratory findings, and histology. Patients with PFIC 1 usually present with cholestasis during the first few months of life.[41] Intense pruritus, hepatosplenomegaly, and portal hypertension

Table 2
Summary of genetic causes, clinical characteristics, and histologic features of progressive familial intrahepatic cholestasis Disorders

Disorder	Gene	Clinical Manifestations	Laboratory Findings	Cancer Risk	Response to sEHC	Histology
FIC1 disease (PFIC 1,Byler disease)	ATP8B1	Severe pruritus Extrahepatic manifestations(diarrhea, pancreatitis, sensorineural hearing loss) Diarrhea and/or hepatic steatosis with progressive fibrosis posttransplant DILI, low-GGT ICP, contraceptive-induced cholestasis	Normal GGt	Not reported	Yes	Bland cholestasis Coarse and granular intracanalicular bile (EM)
BSEP disease (PFIC 2)	ABCB11	Sever pruritus Risk of recurrence following OLT due to anti-BSEP antibodies Cholelithiasis ILI, low-GGT ICP, contraceptive cholestasis	Normal GGT AST and ALT may be more elevated than in other PFIC disorders	HCC Cholangiocarcinoma Pancreatic adeno-carcinoma	Yes (more likely if "mild" mutation such as E297 G or D482 G)	Giant cell hepatitis Amorphous bile (EM)
MDR3 disease (PFIC 3)	ABCB4	Moderate-to-severe pruritus Progressive biliary disease with generally later onset than other PFIC disorders Cholelithiasis (LPAC) DILI, high-GGT ICP, contraceptive-induced cholestasis	Elevated GGT	Cholangiocarcinoma	No	Changes in cholangiocytes Bile duct proliferation

TJP2 disease (PFIC 4)	TJP2	Cholestatic liver disease with characteristics overlapping with FIC1 and BSEP disease Sensorineural hearing loss Low-GGT ICP Cholelithiasis per one report	Normal GGT	HCC	Not reported	Bland cholestasis
FXR disease (PFIC 5)	NR1H4	Early infantile onset with rapid progression to liver failure (coagulopathy with low factor 5 levels and vitamin K refractoriness) Hepatic steatosis posttransplant Biliary stones ICP	Normal GGT	Not reported	Not reported	Giant cell transformation Bile duct proliferation
MYO5B disease (PFIC 6)	MYO5B	Many present as isolated cholestatic liver disease or in patients with MVID May present or worsen following intestinal transplant for MVID Neurologic involvement in a few cases Biliary stones per one report	Normal GGT	Not reported	Yes (very limited data)	Giant cell hepatitis Canalicular and hepatocellular cholestasis
USP53 (PFIC 7)	USP53	Self-limited cholestasis, or episodic BRIC-like course in many Some with excellent response to rifampin Cholelithiasis Sensorineural hearing loss	Normal GGT	Not reported	Not reported	Mild ductular reaction Periportal fibrosis Mild lobular inflammation

Abbreviations: AFP, alpha fetoprotein; BSEP, bile salt export pump; FIC1, Familial Intrahepatic Cholestasis 1; FXR, Farnesoid X Receptor; GGT, gamma glutamyl transferase; HCC, hepatocellular carcinoma; ICP, intrahepatic cholestasis of pregnancy; LPAC, low-phospholipid associated cholelithiasis; MDR3; Multidrug Resistance 3, MYO5B; myosin 5B, PI; pancreatic insufficiency, TJP2; Tight Junction Protein 2, USP53; ubiquitin-specific protease 53.

may develop. Extrahepatic manifestations can include diarrhea, pancreatitis, exocrine pancreatic insufficiency, elevated sweat chloride, pneumonia, and/or sensorineural hearing loss.[23] Growth failure with stunting is more predominant in FIC1 disease than in BSEP or MDR3 disease.[7] Laboratory findings are remarkable for elevated conjugated bilirubin and serum bile acids at presentation, and mildly to moderately elevated aminotransferases in the setting of normal gamma-glutamyl transferase (GGT) levels. Liver biopsy typically reveals bland canalicular cholestasis, and granular "Byler" bile may be noted on electron microscopy.[5,42] Hepatic manifestations may progress to chronic end-stage liver disease requiring transplantation; Van Wessel and colleagues, in the NAPPED consortium, observed that fewer than half of FIC1 patients survived to adulthood with native liver.[41] However, prognosis seems difficult to determine in individuals with this disorder; neither *ATP8B1* mutation nor serum bile acid level at presentation predicted prognosis in FIC1 patients in the NAPPED study. Similarly, responses to sEHC, although generally more favorable in FIC1 disease than in BSEP disease, have been reported as variable among FIC1 disease recipients, even in patients with identical genotype.[15,32,39] Patients with milder phenotypes of FIC1 disease can develop recurrent episodes of cholestasis without progressive liver disease. This is typically referred to as benign recurrent intrahepatic cholestasis or "BRIC" (type 1).[16] Some patients presenting with a BRIC phenotype, however, may evolve to a more persistent and progressive PFIC phenotype.[23] Low-GGT ICP is another manifestation of FIC1 disease. Liver cancer has not been reported in children with isolated FIC1 disease.

Familial intrahepatic cholestasis 1 disease management. Supportive therapies are the cornerstone for managing FIC1 disease.[22] These include medical management of pruritus and, if ineffective, nontransplant surgery (see dedicated discussion on this topic above). Biliary diversion (sEHC) generally has higher success rates in FIC1 disease than in BSEP disease, and it should be strongly considered in lieu of liver transplant in appropriate candidates who do not have cirrhosis, given the potentially severe complications that can develop posttransplant in patients with FIC1 disease (discussed below). Following sEHC, some patients with FIC1 disease have shown a dramatic reduction in serum bile acids and complete resolution of pruritus; many have followed a course of relapsing/remitting cholestasis and pruritus resembling BRIC.[32,35,39] Addressing nutritional deficiencies (screening for fat-soluble vitamin deficiencies and prescribing replacement when required, prescribing medium chain triglyceride (MCT) oil or MCT-enriched formulas), assessing bone mineral density, screening for extrahepatic manifestations of disease, and managing sequelae of portal hypertension and end-stage liver disease are imperative. Liver transplantation is often reserved for patients who develop cirrhosis and end-stage liver disease.[2,5,22] It is important to note that extrahepatic manifestations of FIC1 disease do not improve after transplantation, and diarrhea and/or hepatic steatosis can become significant complications in many patients posttransplant. Diarrhea is likely due to bile acid diarrhea secondary to normalized bile flow into the FIC1-deficient intestine from the healthy transplanted liver and has been shown to improve with bile acid binding resins such as cholestyramine, or with PEBD.[16,43] FGF19 analogs have also been proposed as a putative therapy; this requires further investigation. Additionally, hepatic steatosis may develop in the allograft and may be associated with progressive fibrosis, and, in some cases, cirrhosis, which may necessitate retransplantation.[39,43,44] The precise mechanisms for this are unclear but, as in the case with diarrhea posttransplant, are likely related to restoration of normal bile flow from the transplanted liver into the FIC1-deficient gut and disordered enterohepatic circulation. As is the case for FIC1 disease postliver transplant

diarrhea, sEHC has shown promise in reversing posttransplant hepatic steatosis in FIC1 disease and should be considered. Preventive internal diversion at the time of transplant was successfully performed in one case, although efficacy of this procedure in preventing allograft steatosis requires further study.[44,45]

Bile salt export pump disease: ATP binding cassette subfamily B member11 (OMIM 601847, 605479)

Bile salt export pump disease genetics and pathophysiology. Mutations in the *ATP binding cassette subfamily B member11 (ABCB11)* gene on chromosome 2 result in BSEP disease, also termed PFIC 2. This leads to impaired bile salt transport into the canaliculus and translates into hepatocellular injury and pruritus.[7] A clear relationship between gene mutation severity and disease severity has been demonstrated in BSEP disease, unlike in FIC1 disease.[14] Mutations that result in only partial loss of BSEP function, with p.D482 G and p.E297 G being notable examples, are generally associated with less severe disease (including BRIC, which is characterized by nonprogressive, episodic cholestasis), good response to ursodiol, longer SNL, and more consistent improvement in SNL following sEHC.[14,39] Variants A590 T and R1050 C have also been reported in association with a BRIC phenotype. Conversely, individuals with at least one severe mutation leading to a predicted nonfunctional protein (often frameshift, protein-truncating mutations or large deletions) tend to have shorter SNL, more frequent hepatocellular carcinoma (HCC), and less favorable response to sEHC. Mild or heterozygous mutations may result in other manifestations of this disease including transient neonatal cholestasis.[12,46] Some variants, including the relatively common V444 A, may be associated with drug-induced hepatic cholestasis or with low-GGT ICP.[8,47,48]

Bile salt export pump disease clinical manifestations, laboratory findings, and histology. Patients with BSEP disease typically present during infancy and frequently have progressive chronic liver disease, including pruritus, jaundice, fat-soluble vitamin deficiencies, portal hypertension, and cirrhosis.[31,41] Serum bile acids are elevated but GGT is normal. Giant cell hepatitis is a notable finding on liver biopsy, as well as canalicular cholestasis with amorphous (rather than coarse and granular) bile, and absence of BSEP protein. AST and ALT are more elevated than observed in FIC1 disease, and early progression to advanced liver disease and liver transplantation are reported more frequently than in FIC1 or MDR3 disease.[7,23] Initial presentation with severe bleeding due to vitamin K deficiency, in the absence of other significant stigmata of liver disease, has been reported.[49] Because BSEP protein is expressed exclusively in the liver, deficiency does not present with extrahepatic manifestations, as observed in FIC1 disease.[2,5,16] Patients with BSEP disease are at particular risk for HCC, even in the absence of cirrhosis, and HCC may develop even in very young patients[50] [6]. Cholangiocarcinoma and pancreatic adenocarcinoma have also been reported.[51,52]

Bile salt export pump disease management. Supportive care for BSEP disease is similar to what is recommended for other PFIC disorders, with emphasis on pruritus management, nutrition optimization, fat-soluble vitamin supplementation, and expectant management of complications associated with cirrhosis and portal hypertension.[5,53] Ursodiol is a mainstay of therapy and may have a particularly significant beneficial effect in those with less severe mutations. Screening for hepatic malignancies with tumor markers (including alpha-fetoprotein [AFP]) and abdominal imaging is critical for children with BSEP disease and has been recommended beginning at around 12 months of age.[16] Response to sEHC in BSEP disease depends on degree

of BSEP activity retention, with worse outcomes in patients with absent protein but more consistently good outcomes in those with D482 G and/or E297 G or other less severe mutations. Although liver transplantation is generally curative, especially given that BSEP is expressed exclusively in the liver and that there are no extrahepatic manifestations of BSEP disease, up to 10% of PFIC2 patients develop recurrent liver disease with evidence of anti-BSEP antibodies after liver transplant. This phenomenon has been termed autoimmune BSEP disease, and it has been effectively managed with B-cell depleting antibody therapy (rituximab), IVIG, plasmapheresis, steroids, and/or use of mycophenolate as part of the posttransplant immunosuppression regimen.[54–56]

Multidrug resistance 3 disease: ATP binding cassette subfamily B member 4 (OMIM 602347)

Tight Junction protein 2 disease genetics and pathophysiology. Mutations in the *ATP binding cassette subfamily B member 4 (ABCB4)* gene, on chromosome 7, cause MDR3 disease, also known as PFIC 3. *ABCB4* encodes phospholipid transporter MDR3, which is located on the canalicular membrane of hepatocytes and is critical for bile acid transport.[6,16,41] Mutations in *ABCB4* can lead to defective MDR3 and impaired neutralization of free bile acids, resulting in so-called toxic, low-phospholipid bile with resultant biliary injury.

Multidrug resistance 3 disease clinical manifestations, laboratory findings, and histology. MDR3 deficiency causes low phospholipid-associated cholestasis and/or cholelithiasis (both also sometimes referred to as low phospholipid-associated cholelithiasis [LPAC]). It may manifest with pruritus and jaundice but progressive hepatic fibrosis with portal hypertension is a more salient feature of this disorder compared with FIC1 and BSEP disease.[7] Age of onset is later than observed in FIC1 and BSEP disease, with clinical findings usually first notable in childhood or adolescence rather than during infancy. Unlike in FIC1 and BSEP disease, GGT is elevated in MDR3 deficiency. Hepatic aminotransferases and serum bile acids are commonly elevated, and bilirubin is sometimes elevated. Liver biopsy may demonstrate bile duct proliferation, intraductal stone formation, and giant cell hepatitis.[16] Cholestasis leads to impaired copper excretion in bile and may lead to hepatic copper accumulation, and patients with MDR3 disease may present with hepatic copper overload and clinical features overlapping with Wilson disease.[57–59] *ABCB4* variants may be associated with LPAC, drug-induced liver injury, transient neonatal cholestasis, increased susceptibility to parenteral nutrition-associated cholestasis, and high-GGT ICP, and these disorders may be responsive to ursodiol.[4,8,11,36,47,60,61]

Multidrug resistance 3 disease management. Management focuses on supportive care including pruritus management[41] and ursodiol, which may not only help to alleviate pruritus but also to reduce hepatic fibrosis in some cases; it has even been reported to reverse cirrhosis this disorder.[27] Surgical diversion (sEHC) does not improve liver disease in this disorder, and transplantation remains the only therapy in medically refractory cases with end-stage liver disease or cancer. Screening for cholangiocarcinoma is warranted, as this has been reported.[60]

Tight junction protein 2 disease: tight junction protein 2 (OMIM 615878)

Multidrug resistance 3 disease genetics and pathophysiology. First described in 2014, mutations and loss of function in the *TJP2* gene on chromosome 9 lead to deficiency of TJP2 protein, a cytoplasmic component of the hepatocyte cellular junctions, that prevents leaking of bile between the cells and into the liver parenchyma.[6]

Complete TJP2 deficiency results in severe and progressive liver disease, although other mutations present with milder liver disease or hypercholanemia with fat-soluble vitamin deficiencies.[62,63] There may also be variable disease penetrance, as evidenced in one large family with consanguineous parents, in whom some homozygous adult offspring had developed cirrhosis, whereas others had elevated liver enzymes but no cirrhosis.[64]

Tight junction protein 2 disease clinical manifestations, laboratory findings, and histology. Patients with severe TJP2 deficiency most commonly present during early infancy with low-GGT cholestasis with elevated serum bile acids, pruritus, and progressive liver disease.[62,65] Liver biopsy demonstrates nonspecific findings including intracellular cholestasis. Other variations of TJP2 disease may present as cirrhosis with portal hypertension or as mild liver disease in young adulthood,[64] and heterozygous or homozygous TJP2 mutations in women may result in low-GGT ICP.[8] Cholelithiasis has been reported, as well.[62] Extrahepatic manifestations may include respiratory disease, hearing loss, and/or neurologic symptoms.[66] HCC has been reported in young patients with severe liver disease.[65,67]

Tight junction protein 2 disease management. As for other PFIC disorders, supportive care, including management of fat-soluble vitamin deficiencies and malnutrition, pruritus, and sequelae of portal hypertension when it develops. Early screening for malignancy beginning in the first year of life is recommended, as HCC has been reported in TJP2 deficiency at a very young age.[65,67] Liver transplantation offers definitive treatment of liver disease with or without localized malignancy, although it does not resolve extrahepatic findings. Hepatic steatosis and diarrhea posttransplant have not been reported posttransplant. There have been no reports, at the time of this writing, of outcomes following sEHC in patients with TJP2 disease.

Farnesoid X receptor disease: nuclear receptor subfamily 1, group H, member 4 (OMIM 617049)

Farnesoid X receptor disease genetics and pathophysiology. FXR is a nuclear transcription factor encoded by the *nuclear receptor subfamily 1, group H, member 4 (NR1H4)* gene on chromosome 12 and responsible for regulation of bile salt metabolism. FXR disease, or PFIC 5, results in inappropriate regulation of BSEP and causes a severe variety of neonatal cholestatic liver disease.[6]

Farnesoid X receptor disease clinical manifestations, laboratory findings, and histology. Loss of FXR results in severely impaired bile flow in the neonatal period, coagulopathy refractory to vitamin K supplementation and with evidence of hepatic synthetic dysfunction, and rapid progression to end-stage liver disease.[5] Serum GGT levels are low and serum bile acid levels are elevated. Serum AFP is elevated, often markedly so.[5,68] Liver biopsy shows giant cell hepatitis and hepatocyte ballooning, bile duct proliferation, cholestasis, and absent BSEP and FXR on immunostaining.[2,68] Infants typically present with severe cholestatic liver disease before 2 months of age but onset as late as the second year of life has also been reported. Mutations in NR1H4 may also cause ICP or cholelithiasis.[16]

Farnesoid X receptor disease management. Liver transplant is the definitive therapy for this disorder.[68] Due to its typically rapidly progressive course, diagnosis may not be determined before transplant but outcomes have reportedly been good in the transplant recipients reported in the medical literature thus far. FXR does have extrahepatic expression, and hepatic steatosis has been reported in a few transplant

recipients in the posttransplant period. More data are required to determine prognosis for this complication in children transplanted for FXR disease.

Myosin 5B disease, myosin 5B (MIM 251850)

Myosin 5B disease genetics and pathophysiology. MYO5B, encoded by the *MYO5B* gene on chromosome 18, plays an important role in cell membrane trafficking and localization of BSEP and other canalicular proteins via its interaction with rab11a.[69] With inappropriate canalicular protein localization and hepatocyte membrane polarization, adequate bile acid secretion is impaired and results in hepatotoxicity.[70] Defects in MYO5B historically have been associated with intestinal microvillous inclusion disorder (MVID), which causes severe congenital diarrhea that may necessitate parenteral nutrition and intestinal transplant. In 2013, Girard and colleagues reported a PFIC-like cholestatic liver disease in 8 children out of a cohort of 28 patients with MVID that was apparently unrelated to parenteral nutrition.[71] Subsequently, isolated cholestatic liver disease in patients with MYO5B mutations, but without apparent intestinal disease, was reported in 5 children.[70] The patients in this series had either compound heterozygous or homozygous mutations in MYO5B, all of which were previously unreported in patients with either isolated MVID or MVID with cholestasis. As such, a distinct disorder was identified. Interestingly, MYO5B's role in the development of cholestasis seems to rely solely on its interaction with active rab11a, rather than on its loss of motor function.[69] Thus, although severe (eg, biallelic nonsense or frameshift) mutations in the *MYO5B* gene consistently result in MVID, this genotype–phenotype relationship does not apply to MYO5B liver disease.[69]

Myosin 5B disease clinical manifestations, laboratory findings, and histology. Patients with MYO5B liver disease present with low-GGT cholestasis and elevated serum bile acids, typically in late infancy or early in the second year of life, and may have hepatomegaly, pruritus, and/or pale stools. In patients with MYO5B cholestasis *and* MVID, cholestasis with significant pruritus may worsen or may newly develop following intestinal transplant.[71] AFP levels have been normal in cases reported to-date. Liver biopsy demonstrates canalicular cholestasis and giant cell transformation, with abnormal MYO5B, RAB11a, MDR3, and BSEP immunostaining.[2,5,42,70] Extraintestinal manifestations reported include episodic diarrhea, which resolved after 3 years of age in one infant who had normal duodenal histology, and neurologic symptoms of unclear cause in one child with a normal vitamin E level.[70] Cholelithiasis was reported in a child with MYO5B disease in one case series, with recurrent "mild and self-limiting" diarrhea before 3 years of age, who was not diagnosed with MVID and presumably not treated with parenteral nutrition, although this was not explicitly stated.[72] Cancer has not been reported in MYO5B liver disease at the time of this writing.

Myosin 5B disease management. Management consists of supportive care targeting pruritus, fat-soluble vitamin deficiencies, controlling diarrhea, and optimizing nutrition.[70] Ursodiol and/or rifampin has improved cholestasis and pruritus but episodic cholestasis may occur despite its continuous use. In patients with both MVID and cholestatic liver disease (with onset either before or after intestinal transplant), medical therapy for pruritus has shown inconsistent effectiveness, pruritus may worsen following intestinal transplant, and hepatic fibrosis may progress.[71] As such, it has been suggested that combined intestinal/liver transplant should be considered in patients with MVID and severe cholestatic liver disease who are being considered for intestinal transplant. In small bowel transplant patients with cholestasis and pruritus posttransplant, removal of a failed bowel graft has resulted in complete or partial

remission of pruritus, presumably due, at least in part, to loss of enterohepatic circulation of bile acids.[71] Nontransplant surgery such as partial external biliary drainage (PEBD) may improve cholestasis and alleviate pruritus in medically refractory cases of isolated MYO5B liver disease, as well as in patients with MVID and MYO5B cholestasis, either before or after intestinal transplant.[70,71] For patients with MVID undergoing intestinal transplant, conservation of the gallbladder should be considered so that PEBD can be considered if cholestasis worsens after transplant. Ileal exclusion has effectively alleviated pruritus in MYO5B liver disease but only transiently in at least one case in which it was reported.[71] Combined small bowel and liver transplant for MVID with advanced liver disease have been performed effectively; isolated liver transplant has not yet been reported.

Ubiquitin-specific protease 53 deficiency, ubiquitin-specific protease 53

Ubiquitin-specific protease 53 disease genetics and pathophysiology. USP53 is encoded by the *USP53* gene on chromosome 4. In mice, it has been shown to colocalize and interact with TJP2 and to contribute to tight junction function in the ear.[83] USP53 liver disease was first reported in 2019, in 3 infants from a single family from Saudi Arabia in whom a novel, homozygous truncating mutation in the *USP53* gene was detected by whole exome sequencing.[73] Subsequently, additional cases of cholestatic liver disease in children with biallelic mutations in *USP53* have been reported.[20,62,74,75]

Ubiquitin-specific protease 53 disease clinical manifestations, laboratory findings, and histology. At the time of this writing, 19 cases of USP53 liver disease have been described.[20,74–76] The disorder manifests as low GGT cholestasis, with elevated serum bile acids and modestly elevated aminotransferases. USP53 disease reportedly presents during infancy in most patients but during childhood or adolescence in some. Disease course is self-limiting or episodic in many and may respond well to rifampin.[20,76] Although fibrosis on liver biopsy and splenomegaly were reported in several affected patients in one report, no descriptions yet of complications of end-stage liver disease or significant portal hypertension have been reported for in this disorder. Cholelithiasis was reported in several patients.[20,74,75] Liver biopsy demonstrates periportal fibrosis and mild lobular activity in most, variable lobular and canalicular cholestasis, and ductular reaction. In one series, evidence of cholangiopathy was observed on liver biopsies, and its potential cause, especially in the setting of normal GGT, was discussed. The authors noted that loss of USP53 or TJP2 may cause secondary deficiency of the tight junction protein claudin 1, deficiency of which causes neonatal sclerosing cholangitis, a cholangiopathy. The latter disorder, however, is characterized by high GGT, unlike USP53 and TJP2 deficiencies, and there is no evidence of cholangiopathy in TJP2 deficiency.[74] Autoimmune cause was described as another possible mechanism but no definite evidence for this was described. Extrahepatic manifestations reported included hearing loss in several patients described in 2 of the articles, including one patient who was deaf from birth and later received a cochlear implant.[74,76] Developmental and speech delay was reported in a single patient. None of the patients cited so far have developed cancer or end-stage liver disease. One received liver transplant at 6 years of age for intractable pruritus.[74]

Ubiquitin-specific protease 53 disease management. Recommended medical management for USP53 disease should be similar to the other PFIC disorders, focused on optimizing fat-soluble vitamin levels and nutrition in the setting of cholestasis and associated malabsorption, and relieving pruritus. Ursodiol has been used in many of the patients described thus far, and rifampin has shown excellent benefit in

some, including in one child who developed complete normalization of liver enzymes and bilirubin along with complete resolution of pruritus while taking rifampin.[20,74,76] Another had resolution of disease while taking ursodiol and rifampin, then experienced relapse of pruritus on cessation of these medications, and experienced resolution of pruritus again after restarting rifampin. Nontransplant management of cholestasis and pruritus using sEHC has not yet been reported in USP53 disease.

Other Intrahepatic Cholestasis Genes

Recently, mutations in the lipolysis-stimulated lipoprotein receptor (LSR) gene (encoding LSR) have been reported in 2 patients (in separate publications).[73,77] LSR is a tight junction protein whose complete absence causes liver hypoplasia and fetal death in mice. Both patients presented during the first year of life with low-GGT cholestasis with elevated serum bile acids and severe pruritus that was unresponsive to ursodiol.[73,77] Mutations were biallelic and predicted to be pathogenic in both patients, who had nonprogressive disease course at last follow-up. Both also had mild speech and cognitive delay but hearing deficits were not reported.

Four patients with infantile cholestasis and diarrhea, with biallelic mutations in the Unc-45 myosin chaperone (*UNC45 A*) gene, were described in 2018.[78] GGT was normal in 3 patients but elevated in the fourth as early as 7 days of age; this patient received parenteral nutrition although timing of its initiation and its potential contribution to liver disease was not discussed. Two patients with intractable pruritus had good response to PEBD. Severe congenital diarrhea necessitating parenteral nutrition was present in 3 patients; this persisted to at least 5 years of age in one and resolved in the others. Other associated findings included hearing loss, mild developmental delay, and bone fragility, without evidence of vitamin D deficiency or parathyroid dysfunction in at least one patient.

In another report, homozygous truncating mutations in the *SLC51* B gene (encoding organic solute transporter beta [OSTb]) were identified in 2 siblings with high-GGT cholestasis, fat-soluble vitamin deficiencies, and chronic diarrhea (with normal intestinal histology) with infantile onset.[79] Serum bile acid levels were low. OSTb, similar to ASBT, is an intestinal bile acid transporter; its dysfunction, in these cases, presumably caused disruption of enterohepatic circulation of bile acids, with resultant bile acid and fat-soluble vitamin malabsorption, and cholestasis.

SUMMARY

PFIC represents a heterogenous group of monogenic disorders stemming from defects in bile acid transport/secretion.[3,5] The availability of rapid genetic diagnostics has yielded further understanding of defects impacting bile acid trafficking and contributing to the clinical phenotype of cholestasis and progressive liver disease,[2,6,80] and it is expected that the number of identified PFIC disorders will continue to grow. The mainstay of treatment remains supportive, including management of pruritus, malnutrition, fat-soluble vitamin deficiencies, and sequelae of end-stage liver disease, as well as cancer screening—particularly in BSEP, TJP2, and MDR3 disease. Liver transplant is a definitive treatment of end-stage liver disease, malignancy confined to the liver, and medically refractory disease. Nontransplant surgery (such as PEBD) may be successful in certain types of PFIC (MDR3 disease is a notable exception) and may be especially suited to patients with FIC1 disease, in whom many have a good response, and in whom liver transplant should be carefully considered due to the possibility of chronic diarrhea and/or hepatic steatosis with progressive fibrosis posttransplant in this disorder. iBAT inhibitors are a novel class of

pharmacologic therapy that interrupt enterohepatic circulation and reduce serum bile acids and pruritus.[22] More studies are needed to determine effects on the progression of liver disease. Other prospective future treatments, such as gene therapy or hepatocyte transplantation, could potentially prove effective. These would be particularly desirable for FIC1 disease, in which liver transplant is often fraught with postoperative complications but residual cancer risk in BSEP and TJP2 diseases may render new nontransplant therapies for these disorders less attractive.[31,81,82]

CLINICS CARE POINTS

- Familial intrahepatic cholestasis 1 (FIC1), bile salt export pump (BSEP), tight junction protein 2 (TJP2), myosin 5B (MYO5B), farnesoid X receptor, and ubiquitin-specific protease 53 disease are all characterized by defects in canalicular bile transport and/or secretion with low-normal gamma-glutamyl transferase (GGT) level (usually <100 IU/L), whereas patients with multidrug resistance 3 (MDR3) disease typically have elevated GGT associated with biliary injury due to low-phospholipid bile.
- Patients with progressive familial intrahepatic cholestasis (PFIC) may develop jaundice and/or hepatic fibrosis, and pruritus is a significant disease complication. Lack of adequate intestinal bile may cause malabsorption of fat and fat-soluble vitamins with consequent malnutrition, low bone mineral density, vitamin-K responsive coagulopathy, and/or complications of vitamin A or E deficiency.
- BSEP and TJP2 disease are associated with cancer risk, and affected individuals should undergo regular cancer screening, including laboratory evaluation and imaging, from a young age.
- Biliary diversion surgery (enterohepatic circulation; PEBD, PIBD, or ileal exclusion) may alleviate symptoms and/or slow disease progression in FIC1, BSEP, and possibly MYO5B deficiency but it should not be performed in patients with cirrhosis and/or MDR3 deficiency and may be less effective in some patients with severe (no residual protein function) mutations.
- Liver transplant is recommended in the setting of end-stage liver disease, refractory pruritus, and/or cancer confined to the liver but should be approached with caution in FIC1 disease given the possibility of posttransplant diarrhea and/or hepatic steatosis, and autoimmune BSEP disease should be screened for in posttransplant BSEP-deficient patients.
- Odevixibat, an ileal apical sodium-dependent intestinal bile acid transporter inhibitor, medically impairs enterohepatic circulation and has proven promising as a new and safe therapy for pruritus in the PFIC disorders.

DISCLOSURE

The authors have nothing to disclose.

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