Molecular Advances in Cholestatic Liver Diseases

Raima Memon, MD, and Romil Saxena, MD†*

Abstract: The list of genetically defined causes of cholestatic liver diseases continues to expand; it currently includes mutations affecting bile acid synthesis, basolateral and apical membrane transporters, bile duct development, canalicular tight junctions, and bile acid conjugation, among others. The most frequently identified mutations in large multi-institutional studies of cholestasis occur in *JAG1, ATP8B1, ABCB11, ABCB4, SERPINA1*, and *CFTR*. Mutations in *JAG1*, *SERPINA1,* and *CFTR* cause Alagille syndrome, alpha-1 antitrypsin deficiency, and cystic fibrosis, respectively. Mutations in *ATP8B1*, *ABCB11,* and *ABCB4* cause a spectrum of diseases that range from the episodic, nonprogressive benign recurrent intrahepatic cholestasis and intrahepatic cholestasis of pregnancy to the severe and rapidly progressive familial intrahepatic cholestasis. These cholestatic disorders present a wide range of symptoms and overlapping clinical features. However, in contemporary practice, diagnosis is often easily and rapidly established by clinically available comprehensive gene panels. In addition to diagnosis, these panels also aid in the discovery of novel genes or variants as potential causes of cholestasis. Genetic mutations may also be responsible for drug-induced cholestasis, as the liver plays a vital role in metabolism of drugs and xenobiotics. Uptake into hepatocytes and elimination into the bloodstream or bile of drugs and xenobiotics involve transporters across the basolateral and apical hepatocellular membranes, respectively. Therefore, mutations in any of the transporters lead to impaired metabolism and/or elimination of these substances. Furthermore, a large number of drugs and xenobiotics have a transcriptional or functional inhibitory effect on transporters such as BSEP and MDR3, setting the stage for the all-too-common drug-induced cholestasis.

Key Words: alagille, canalicular transporters, canalicular membrane, intrahepatic cholestasis, pruritis

(*Adv Anat Pathol* 2025;32:375–386)

The functions of bile are manifold: it supplies bile salts necessary for absorption of fats and fat-soluble vitamins, provides bicarbonate for neutralization of gastric acid in the intestine, and serves as an essential route for elimination of bile-soluble products of metabolism. Cholestasis, or impairment of bile flow, therefore leads to malabsorption and deficiency of fats and fat-soluble vitamins, as well as accumulation of metabolites, including environmental toxins and xenobiotics.

The causes of cholestasis are numerous, ranging from infections (viral, bacterial, parasitic, spirochetes) to toxins (drugs, total parental nutrition, herbal products) and biliary obstruction (biliary atresia, biliary sludge, cholelithiasis, choledochal cyst) to cardiovascular or circulatory disorders and malignancy. However, the most notable advances in recent decades have been in the discovery of genetic mutations in the pathogenesis of cholestasis. These discoveries have additionally facilitated a nuanced understanding of the very complex molecular process of bile formation and secretion.

Components of bile are made by hepatocytes and transported into the bile canaliculus where they combine in appropriate proportions to form bile. The bile canaliculus is formed by the apical membranes of 2 adjacent hepatocytes. The bile canalicular membrane hosts the ATP-dependent superfamily of ABC transporters, including multidrug resistance (MDR) proteins, bile salt export pump (BSEP), and multidrug resistance-associated (MRP) proteins. Molecules residing in the tight junctions of the bile canaliculus seal it off from the adjacent sinusoidal bloodstream; these molecules include occludin, claudin, and zona occludin (ZO-1).

There is now a large and continuously expanding list of genetically defined causes of cholestatic liver disease.[1] These include disorders of bile acid synthesis (*AKR1D1, AMACR, CYP7B1, HSD3B7, CYP7A1, and CYP27A1*), defects in canalicular membrane transporters (*ABCB11, FXR, FIC1, ABCB4*), disorders of bile duct development (*JAG1, NOTCH2, PKHD1),* disorders of tight junctions (*CLDN1, TJP2, MYO5B*), secretion defects (*CFTR*), disorders in protein folding (*SERPINA1*), disorders of bile acid conjugation (*BAAT* and *SLC27A5*), and fatty acid oxidation defects (*SCAD* and *LCAD*).[2–4]

These cholestatic disorders present a wide range of symptoms, posing a significant diagnostic challenge as many exhibit overlapping clinical features. Through careful examination of symptoms, imaging, laboratory markers, and histopathology, the potential causes of cholestatic liver disease can be narrowed down. However, in contemporary practice, diagnosis is often easily and rapidly established by clinically available comprehensive gene panels, which can detect all known gene variants, including those associated with *JAG1, NOTCH2, ATP8B1, ABCB11, ABCB4, SERPINA1, ABCC2, ARK1D1, BAAT, CFTR, CLDN1, and SCL24A1*. In addition to diagnosis, these panels also aid in the discovery of novel genes or variants as potential causes of cholestasis.[1,5–7]

Of the numerous genes, the most frequently identified in large multi-institutional studies of cholestasis are *JAG1, ATP8B1, ABCB11, ABCB4, SERPINA1*, and *CFTR*.[1,7–9] Mutations in *JAG1*, *SERPINA1,* and *CFTR* cause Alagille syndrome, alpha-1 antitrypsin deficiency (AATD), and cystic fibrosis (CF), respectively. Mutations in *ATP8B1*, *ABCB11,* and *ABCB4* cause a spectrum of diseases that range from the episodic, nonprogressive benign recurrent intrahepatic cholestasis (BRIC) and intrahepatic cholestasis of pregnancy (ICP) to the severe and rapidly progressive familial intrahepatic cholestasis (PFIC). Mutations in *SERPINA1* causing AATD are not directly involved in bile formation but are commonly identified during investigation of clinical cholestasis. This review focuses on the molecular

From the *Roswell Park Cancer Center; and †Department of Pathology, Emory University School of Medicine, Buffalo, NY.
The authors have no funding or conflicts of interest to disclose.
Reprints: Romil Saxena, MD, Department of Pathology, Emory University School of Medicine, 1364 Clifton Road NE, Room H-182A, Atlanta, GA 30322 (e-mail: Romil.Saxena@emory.edu).
All figures can be viewed online in color at www.anatomicpathology.com.

DOI: 10.1097/PAP.0000000000000502

basis, pathophysiology, and clinical aspects of these more common disorders.

ALAGILLE SYNDROME

Alagille syndrome (ALGS) is an autosomal dominant multisystem disorder with a reported incidence of 1:30,000 to 1:100,000. ALGS is caused by mutations in genes of the NOTCH signaling pathway, which is composed of 4 NOTCH receptors (NOTCH 1, 2, 3, and 4) and 5 ligands (JAG1, JAG2, DELTA-LIKE 1, 3, and 4). Both JAG and NOTCH are transmembrane proteins with extracellular domains. Binding of ligand to receptor leads to transcriptional activation of downstream genes leading to a diverse array of cellular processes that determine cell fate and differentiation during embryonic development[10–12] and is particularly vital for development of bile ducts. Most ALGS patients carry a heterozygous mutation in *JAG1* located on 20p12. Approximately 70% of these mutations are protein-truncating, most commonly frameshift, followed by nonsense, splice site, and gross deletion. Approximately 1% to 2% of patients carry mutations in *NOTCH2*, located on chromosome 1p13.[13–17]

Liver disease is a major presenting feature of ALGS and varies from mild asymptomatic elevation of liver enzymes to severe cholestatic disease requiring liver transplantation (LT). Symptomatic patients present with jaundice and pruritus, the latter can be debilitating enough to cause self-mutilation and often represents the primary indication for LT. Patients demonstrate variable combinations and severity of extrahepatic manifestations, including peripheral pulmonary stenosis, butterfly vertebrae, posterior embryotoxon, solitary or ectopic kidney, and "Alagille facies," which is characterized by a prominent forehead, deep-set eyes, pointed chin, and flat face with prominent ears. Laboratory tests show serum elevations of conjugated bilirubin, alkaline phosphatase, gamma-glutamyl transferase (GGT), and bile acids.[18–21] The gallbladder is absent in almost a third of patients on imaging. Genetic testing by Sanger sequencing of all 26 exons of JAG1 identifies 85% of patients with ALGS; if no mutations are found, sequencing of 34 exon of NOTCH2 is recommended.

Histologically, the liver shows canalicular and cellular cholestasis, which begins in perivenular (zone 3) areas of the hepatic lobule (Fig. 1A). Kupffer cells may be laden with bile. Portal tracts may lack interlobular bile ducts, leading to the finding of "unpaired arterioles," ie, presence of hepatic arterioles without accompanying bile ducts. Definitive diagnosis requires the absence of bile ducts in at least 50% of portal tracts in a biopsy that contains at least 10 complete portal tracts; ALGS has therefore also been referred to as syndromic paucity of intrahepatic bile ducts. Portal and

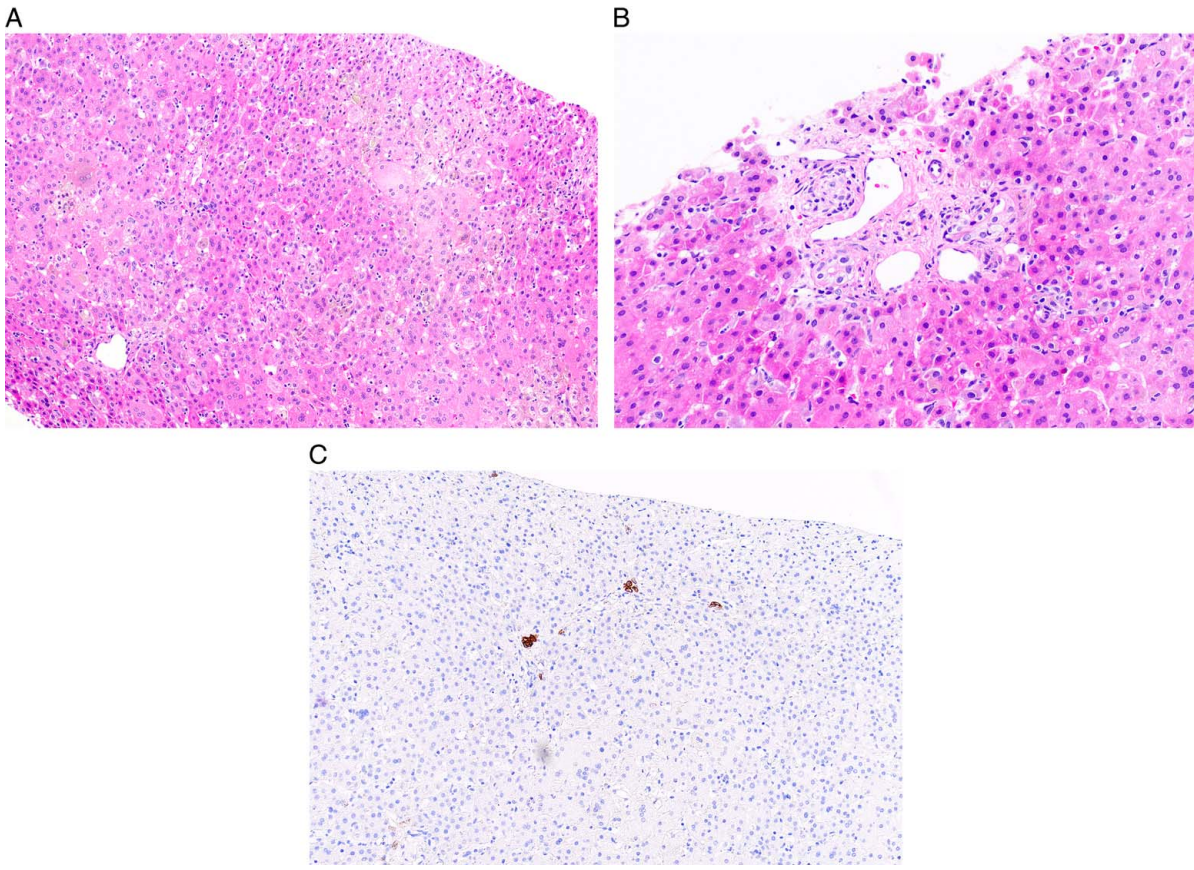

FIGURE 1. A, Alagille syndrome with severe and extensive cellular and canalicular cholestasis. Multinucleated giant hepatocytes containing bile pigment are seen. Bile-laden Kupffer cells are present. Two barely visible portal tracts (upper right and lower left) lack bile ducts. B, A larger portal tract contains a bile duct showing epithelial damage characterized by irregular nuclei and cytoplasmic vacuolation; there is no portal inflammation. C, Immunohistochemical stain for K7 highlights a portal tract without a proper bile duct and g small clusters of residual cholangiocytes.

lobular inflammation, as well as ductular proliferation, are minimal. In the early phases of the disease, bile ducts may still be present in portal tracts, although they show biliary epithelial damage. The latter is characterized by vacuolation of cholangiocytes, loss of nuclei, apoptosis, and presence of lymphocytes within the biliary epithelium (Fig. 1B). Similarly, mild ductular proliferation may be present in the early phase of the disease, but in contrast to biliary atresia, is never marked. An immunohistochemical stain for the biliary keratin, K7, is useful in identifying portal tracts without bile ducts (Fig. 1C), and the stain may further highlight biliary metaplasia of hepatocytes. Portal and periportal fibrosis eventually ensues, leading to bridging fibrosis, albeit the pace of progression is variable and not rapidly progressive. Hepatocellular carcinoma has been reported in $<1\%$ of patients with ALGS.

PROGRESSIVE FAMILIAL INTRAHEPATIC CHOLESTASIS

Progressive familial intrahepatic cholestasis (PFIC) is a group of autosomal recessive disorders that cause severe and rapidly progressive cholestatic disease in infancy and early childhood. Six distinct disorders named PFIC1-6 have been characterized so far. PFIC1-3 are caused by mutations in genes encoding membrane transporters located on the bile canalicular membrane. Being more recent discoveries, PFICs 4-6 are less well characterized but are included in this article to showcase the mutational landscape of intrahepatic cholestasis and the complex pathophysiology of bile formation.[22] A characteristic feature of PFICs (except PFIC-3) is the failure of serum GGT to rise proportionately with serum bilirubin. These disorders are therefore also referred to as "low-GGT cholestasis" (low relative to serum bilirubin), and an early observation was the bad prognosis of low-GGT cholestasis.[23–25]

Progressive Familial Intrahepatic Cholestasis-1

The disease now designated as PFIC-1 has a storied history, being known for long as Byler disease in the Amish and as Greenland familial cholestasis in the Inuit of Greenland and Canada.[10,26] Identification of the disease-causing gene mutations has facilitated identification of PFIC-1 outside these restricted demographics, particularly in areas with high incidence of consanguinity. PFIC-1 affects 1 in 50 to 100,0000 births.

PFIC-1 is an autosomal recessive multisystem disorder caused by mutations in ATPase phospholipid transporting-8B1 gene (*ATP8B1*), located on chromosome 18, that encodes for FIC1 protein. In the liver, FIC-1 resides on the bile canalicular membrane and serves to flip phosphatidylserine from the canalicular aspect of the apical membrane to its hepatocellular aspect.[11] FIC1 protein is also found in several nonhepatic tissues including stomach, small intestine, bladder and pancreas, explaining the extrahepatic manifestations of PFIC-1.[12,27,28]

The exact mechanism whereby FIC1 deficiency leads to cholestasis is uncertain, but is probably related to membrane destabilization and cellular damage due to deficient translocation of phospholipids. Bile formation is further compromised by a negative feedback loop initiated by FIC1 deficiency, which leads to downregulation of farsenoid X receptor (FXR) with subsequent downregulation of the bile salt export pump (BSEP) in hepatocytes as well as decreased bile acid reabsorption through ileal sodium-dependent bile acid transporter (ISBT) in the intestine.[29]

PFIC1 presents in infants with jaundice, pale diarrheal stools, and failure to thrive. Malabsorption of fats and fat-soluble vitamins leads to short stature, malnutrition, coagulopathy, osteodystrophy, and hyperparathyroidism. Pruritus is often debilitating and may lead to self-mutilation. Patients demonstrate extrahepatic manifestations in varying degrees and combinations, including sensorineural deafness, recurrent pancreatitis leading to pancreatic insufficiency, chronic respiratory diseases, and delayed sexual development. Laboratory tests show conjugated hyperbilirubinemia, normal to low-GGT levels, high serum conjugated bile acids, and mildly elevated transaminases. The concentration of bile salts in bile is low. The liver disease progresses rapidly to cirrhosis with worsening of symptoms, hepatosplenomegaly, portal hypertension, and coagulopathy.

Histologically, PFIC-1 shows bland canalicular cholestasis; inflammation, necrosis, or ductular proliferation are not prominent features (Fig. 2A). The bile ducts may show epithelial damage, but are generally present in portal tracts. Periportal and/or perivenular fibrosis is present in a perisinusoidal pattern early in the disease. It progresses rapidly to portal-portal and portal-central bridging fibrosis (Fig. 2B). Immunohistochemical stains show extensive biliary metaplasia of hepatocytes (positivity of hepatocytes for K7) (Fig. 2C), weak or absent canalicular expression of BSEP, and retained expression of MDR3.[30]

Progressive Familial Intrahepatic Cholestasis-2

Progressive familial intrahepatic cholestasis type 2 (PFIC 2) has an incidence of 1 in 50,000 to 100,000 and is caused by mutations in the *ABCB11* (ATP Binding Cassette Subfamily B Member 11) gene located on chromosome 2q31.[31] This gene encodes BSEP, which is localized on the bile canalicular membrane and transports monovalent bile salts out of hepatocytes into the bile canaliculus. Bile salts are the major component of bile and attract water in the canaliculus, facilitating bile flow. Absent or impaired export of bile salts out of hepatocytes causes accumulation within hepatocytes causing cellular damage, and impairs bile flow due to low volume in the canaliculus. BSEP is expressed exclusively in the bile canaliculus with no extrahepatic tissue of expression.[11]

Over 200 mutations in *ABCB11* have been identified causing PFIC-2. Of these, 2 common mutations (p.D482G and p.E297G) have relatively milder phenotype due to residual BSEP function. A severe phenotype has been shown in patients with predicted protein truncated mutations (PPTMs).[32]

Similar to PFIC-1, patients present in early infancy with jaundice, diarrheal pale stools, and failure to thrive. As with PFIC-1, pruritus is debilitating and leads to self-mutilation. Jaundice is, however, more severe, disease progression more rapid, and there is a disproportionately high risk of malignancy; both hepatocellular carcinoma and cholangiocarcinoma have been reported.[33–35] Unlike PFIC-1, there are no extrahepatic manifestations. Laboratory findings are also similar to PFIC-1 with hyperbilirubinemia, low or normal GGT, elevated serum transaminases and bile acids, and coagulopathy. Transaminitis is higher than that seen in PFIC-1. Concentrations of bile acids in bile are low.

Histologic findings are those of neonatal giant cell hepatitis with multinucleated giant hepatocytes, cellular and canalicular cholestasis, mild ductular reaction, damaged but

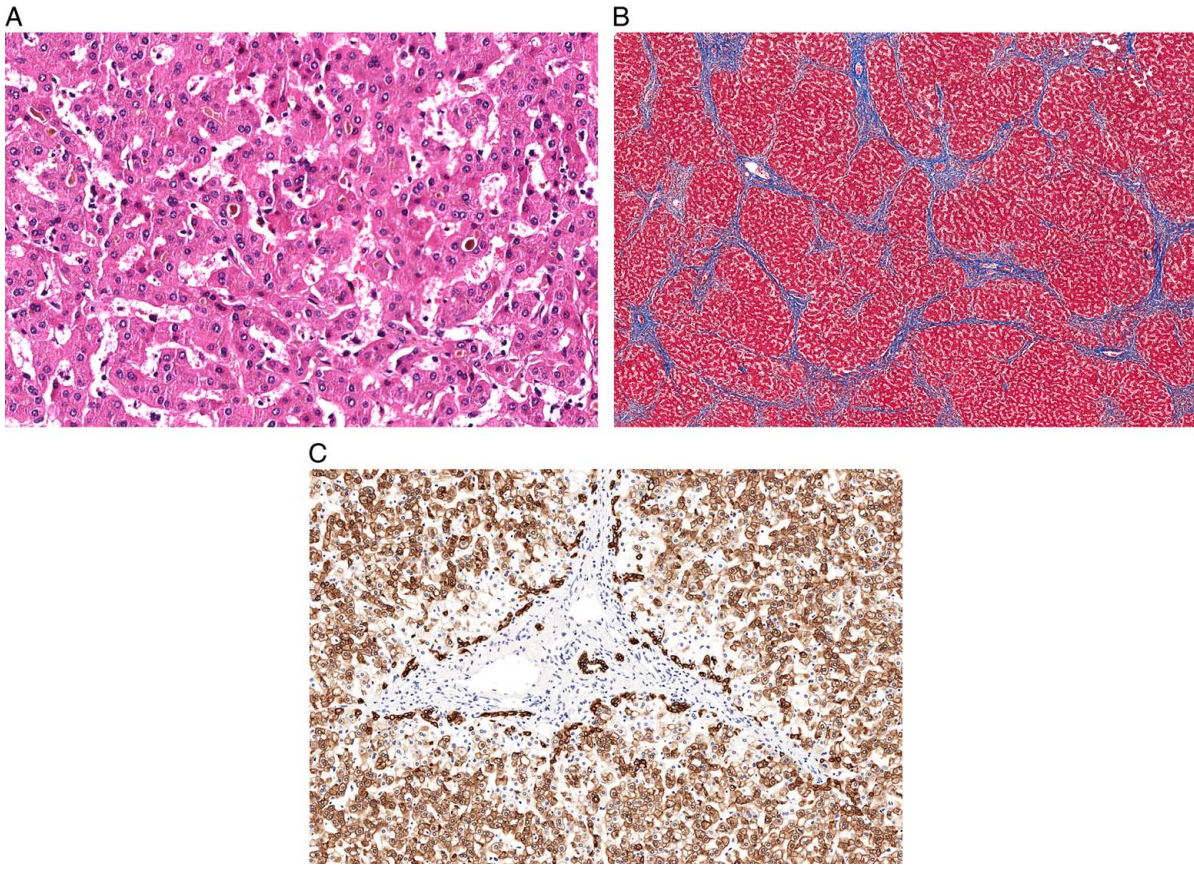

FIGURE 2. A, Liver explanted for PFIC-1 showing extensive canalicular cholestasis in the form of bile plugs within the canaliculi. There is no inflammation or necrosis. B, Trichrome stain shows extensive portal-portal and portal-central bridging fibrosis in the explanted liver. C, Immunohistochemical stain for K7 shows extensive biliary metaplasia of hepatocytes. The interlobular bile duct is present and mild ductular proliferation is seen at the edge of the portal tract.

retained bile ducts, and portal fibrosis. The disease progresses rapidly with marked hepatocellular damage, marked ductular reaction, and progressive fibrosis that leads to biliary-type cirrhosis. There is diffuse giant cell transformation of hepatocytes, which are multinucleated with pale, wispy cytoplasm and abundant intracellular bile (Fig. 3A, B). Immunohistochemical stain for BSEP (bile salt export pump) demonstrates an absence of the characteristic canalicular staining pattern.[30,36]

Progressive Familial Intrahepatic Cholestasis-3

PFIC-3 is caused by the mutations in the *ABCB4* gene, located on chromosome 7q21, which encodes for multidrug resistence-3 (MRD3) glycoprotein.[11] MDR3 is located on the bile canalicular membrane and functions to translocate phospholipids (particularly phosphatidylcholine) from the hepatocellular aspect of the canalicular membrane to its canalicular aspect. The phospholipids are chelated by bile acids to form micelles, which sequester the bile acids and protect the canalicular membrane from the detergent action of bile salts, which are present in a high concentration in the bile canaliculus. When mutated, MRD3 cannot transport phospholipids into the bile canaliculus leading to abnormal micelle formation and an excess of insoluble bile salts in the biliary canaliculi, which cause membrane and cellular damage.

Several different types of mutations in the *ABCB4* gene have been found, which confer varying degrees of disease severity.[37] In one third of patients, the mutation results in truncated protein leading to complete loss of MDR3 expression whereas in the remaining two thirds, missense mutations result in decreased amounts of MDR3 protein.

PFIC-3 presents at a later stage in life compared with PFIC-1 and PFIC-2. Patients have high gamma-glutamyl transferase (GGT) and alkaline phosphatase, and the clinical phenotype ranges from transient cholestasis to episodic cholestasis, gallstones, and biliary cirrhosis.[38] In cases of early onset disease, patients present with jaundice, pruritis, portal hypertension, growth restriction, and learning disabilities.

Histologically, the liver shows a "biliary" process characterized by ductular proliferation and an associated mild inflammatory infiltrate. Canalicular and cellular cholestasis is usually mild (Fig. 4A). Periportal fibrosis is present, which eventually progresses to bridging fibrosis and cirrhosis (Fig. 4B). On immunohistochemistry, there is absent canalicular staining for MDR3,[39] although in cases with a functionally abnormal protein, immunohistochemical staining might be retained.[30]

Progressive Familial Intrahepatic Cholestasis-4

PFIC-4 is a relatively new variant described by Gumbiner and colleagues in 1991. It is caused by mutations

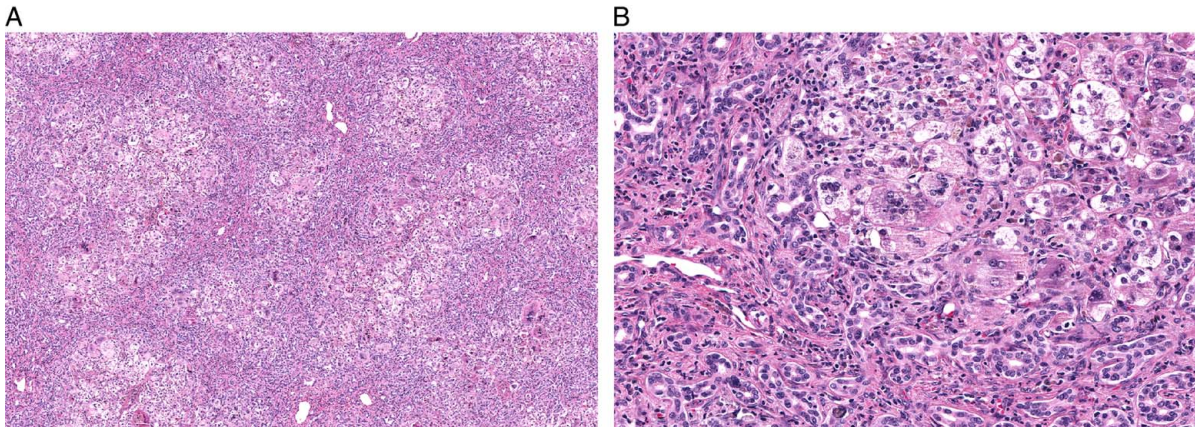

FIGURE 3. A, PFIC-2 showing end-stage liver disease with micronodular cirrhosis, marked hepatocellular damage and biliary proliferation B, High power shows enlarged, markedly damaged hepatocytes containing multiple nuclei, intracellular bile, and coarse granular cytoplasm. Marked ductular proliferation is present in the fibrous septa between nodules (A, B). Courtesy of Dr. Raffaella Morotti, Yale University. Please see this image in color online.

in the tight junction protein 2 (*TJP2*), also known as zona occludens2 (ZO-2) located on chromosome 9q21.[40] TJP2 protein is found in various organs like liver, respiratory system, and central nervous system.[41,42]

In the liver, ZO-2 helps maintain cell adhesion by connecting claudin proteins to the actin cytoskeleton of the cell. Mutations in the *TJP2* gene prevent claudin1 from properly localizing to the membrane, leading to a breakdown in membrane integrity, backflow of toxic bile acids into hepatocytes, and progressive cholestasis. Homozygous *TJP2* mutations result in complete absence of TJP2 protein, while missense and frame deletions allow for partial protein expression and milder disease. No mutations in claudin2 have been reported so far.[42,43]

The clinical presentation of this condition is highly variable, ranging from mild recurrent jaundice to severe liver failure and end-stage liver disease. Laboratory testing typically reveals low to normal levels of the enzyme gamma-glutamyl transferase. In addition to liver-related symptoms, patients may also experience extrahepatic manifestations, such as respiratory and neurological issues. PFIC-4 carries a high risk of developing hepatocellular carcinoma.[44]

Progressive Familial Intrahepatic Cholestasis-5

PFIC-5 is caused by mutations in *NR1H4* located on chromosome 12q23; only 10 cases of PFIC-5 have been reported so far.[45] This gene encodes for the nuclear receptor, farnesoid X receptor (FXR), the master regulator of bile acid metabolism, which is expressed in both the liver and intestines. FXR is involved in the expression of both BSEP and MDR3 and promotes bile acid secretion.[46]

Unique features of PFIC-5 include early onset coagulopathy independent of vitamin K levels, and elevated serum alpha-fetoprotein. As with all PFICs, levels of serum GGT are normal to low. Reported histologic findings include intralobular cholestasis, bile ductular reaction, giant cell formation, and advanced fibrosis.

Progressive Familial Intrahepatic Cholestasis-6 (PFIC Associated with MYO5B Defects)

PFIC-6, also known as PFIC associated with MYO5B defects, is caused by mutations of *MYO5B* gene located on chromosome 18q21.1, which encodes for an actin-associated molecular motor protein called MYO5B.

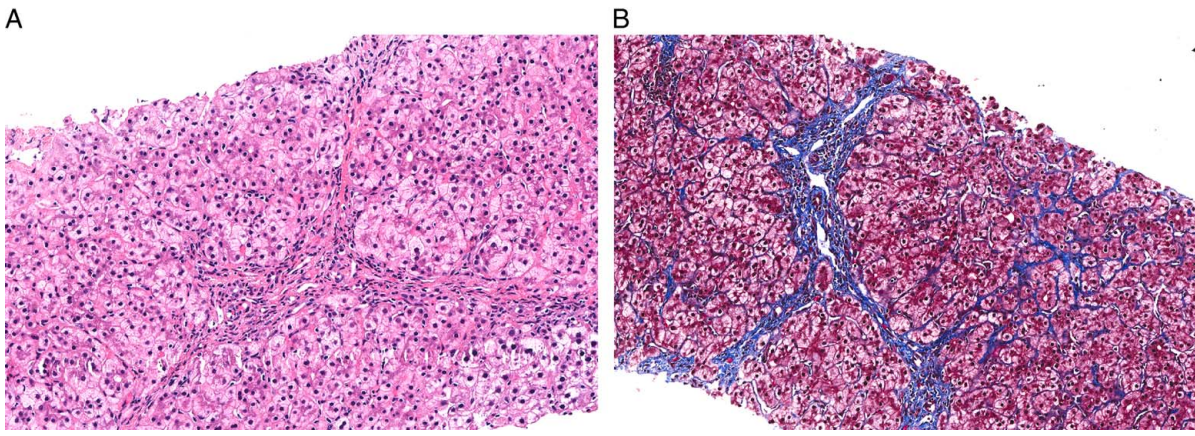

FIGURE 4. A, PFIC-3 showing "biliary" morphology with mild ductular proliferation, preserved interlobular bile duct, and periportal fibrosis. B, Trichrome stain highlights delicate periportal and perisinusoidal fibrosis with thin bridging septa. Please see this image in color online.

MYO5B is expressed in multiple tissues, in which it interacts with RAS-related GTP-binding protein 11A (RAB11A) to maintain polarization of epithelial cells. Disrupted polarity prevents localization of BSEP to the canalicular membrane in hepatocytes, and causes microvillous inclusion disease (MVID) in enterocytes. The clinical phenotype varies with the severity of the mutation. Less severe mutations cause cholestatic liver disease but do not affect intestinal functionality. More severe mutations cause microvillous inclusion disease, causing intractable diarrhea in infancy. This leads to decreased intestinal absorption of bile acids and therefore decreased delivery of bile acids via enterohepatic circulation to the liver, thus preventing cholestasis in the also-deficient liver.[47–49]

Patients with PFIC6 present either with isolated cholestasis, MVID, or both. Jaundice, pruritus, and hepatomegaly are common clinical manifestations. Laboratory tests demonstrate hyperbilirubinemia, low to normal GGT levels, and mild transaminitis. Immunohistochemical stain shows aberrant submembranous localization of BSEP to the cytoplasm below the bile canalicular membrane, instead of being present on the surface of the membrane.

BENIGN RECURRENT INTRAHEPATIC CHOLESTASIS

Benign recurrent intrahepatic cholestasis (BRIC) is an autosomal recessive disorder, which is caused by the same genes that cause PFIC-1 and PFIC-2. BRIC-1 is caused by mutation in *ATP8B1* and BRIC-2 is caused by mutation in *ABCB11*. However, in contrast to PFICs, mutations causing BRIC allow expression of the protein, albeit in reduced amounts.[50,51] Thus, patients with BRIC are asymptomatic till the protein deficiency is unmasked by a stressor.[52] This leads to the episodic nature of cholestasis and associated symptoms, characteristic of BRIC.[53]

BRIC presents as recurrent episodes of jaundice accompanied by intense pruritus, anorexia, nausea, vomiting, and weight loss. The disease can cause marked exacerbation of these symptoms; however, patients remain in good health between episodes. The attacks usually last for a few weeks but can persist up to a few years. Episodes may be triggered by drugs/medications or infections; upper respiratory infections are the commonest. The trigger may not always be clinically obvious.[54] The liver biopsy shows centrilobular canalicular cholestasis, with formation of biliary rosettes and prominent bile-laden Kupffer cells (Fig. 5). There is no accompanying inflammation, necrosis, bile duct loss, or ductular proliferation. Laboratory tests show elevated serum conjugated bilirubin and alkaline phosphatase, whereas GGT and transaminases are generally normal.[55]

INTRAHEPATIC CHOLESTASIS OF PREGNANCY

Intrahepatic cholestasis of pregnancy (ICP) has been known for long, and suspected to be an inherited disease due to clustering in families and ethnic groups, such as people of the Chilean Andes. The molecular genetic basis has been elucidated in recent decades, with some of the same genes involved in PFIC being implicated in its pathogenesis. ICP results from mutations in *ABCB4* and *ABCB11* involved in flopping and flipping phospholipids, respectively, across the bile canalicular membrane. Mutations in *ABCC2* encoding for MRP2 are associated with the development of ICP in South American women. Less commonly, mutations in

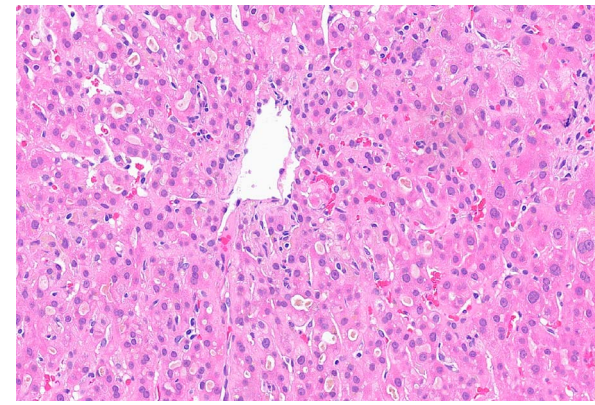

FIGURE 5. Benign recurrent intrahepatic cholestasis with extensive bile plugs within dilated canaliculi present predominantly around the central vein. There is no inflammation or necrosis. Please see this image in color online.

ATP8B1 and *NR1H4* have been associated with the development of ICP. Patients are heterozygous for the mutation allowing expression of the protein, albeit in reduced amounts. Patients thus remain asymptomatic till the protein deficiency is unmasked by pregnancy. Higher incidences in the third trimester and with twin pregnancies point to estrogen as a major player in the pathogenesis; interactions between the estrogen receptor and FXR are known to inhibit expression of BSEP and MRP2.[56–58] In a sense, ICP is but BRIC unmasked or triggered specifically by estrogen excess during pregnancy.

ICP is characterized by onset of severe pruritus during pregnancy with elevated liver enzymes, and normalization of symptoms and liver enzymes following delivery. Pruritus typically starts in the third trimester and first affects palms and soles.[59,60] In ~10% of cases, pruritus is accompanied by icterus. Some patients may experience steatorrhea with decreased absorption of fat-soluble vitamins, leading to weight loss and requirement for vitamin supplementation. A characteristic feature of ICP is spontaneous resolution of symptoms within 2 to 3 weeks of delivery. Serum levels of total bile acids are a sensitive test for diagnosis of ICP. In healthy pregnant women, bile acid levels are slightly high but do not exceed 10 micromoles/L. Studies have shown risk to fetal development at serum bile acid concentration >40 micromoles/L. Increase in serum liver enzymes, specifically ALT of about 2 to 15-fold, has also been observed in ICP.[61,62]

Biopsy does not have a role in the diagnosis or management of ICP; however, it may be performed if the clinical diagnosis is doubtful or if there is suspicion of underlying liver disease. The histologic findings in uncomplicated ICP are those of bland cholestasis with canalicular cholestasis unaccompanied by inflammation, necrosis, bile duct damage, bile duct loss, or fibrosis.

CYSTIC FIBROSIS

Cystic fibrosis (CF) is an autosomal recessive multisystem disease caused by mutations in the gene for cystic fibrosis transmembrane conductor regulator (*CFTR*), which encodes the chloride channel expressed on secretory and absorptive cell surfaces. The prevalence is high in Caucasian populations, affecting between 1:2000 and 1:4000 births of European ancestry[63]; the carrier rate of the *CFTR* gene in

Caucasians is 1:20. The incidence is lower in African-American infants and rare in Asians.

More than 2000 alleles of *CFTR* have been described, several hundred of which are disease-causing. Approximately half of the protein abnormalities are caused by substitution of a single amino acid [phenylalanine residue at position 508 (ΔF508)], and ~40% by nonsense, frameshift and splicing mutations. Large rearrangements of *CFTR* and promoter abnormalities are far less common. The genetic basis remains unclear in ~6% of cases.[64,65]

In the liver, CFTR is expressed on the apical membranes of cholangiocytes; mutations lead to decreased or absent transport of chloride and bicarbonate ions into the biliary system, increasing the viscosity of bile, decreasing bile flow and leading to biliary obstruction and cholangitis. Resultant cell injury and inflammation lead to progressive fibrosis.[64,66]

Liver disease and symptoms may remain silent for many years in the early stages of the disease and patients may present with portal hypertension as a first sign of chronic liver disease.[50,67] The liver disease may be overshadowed by involvement of the lungs and respiratory tract (recurrent infections), pancreas (recurrent pancreatitis and CF-related diabetes) or urinary system (calculi). Serum liver tests may be elevated with serum GGT elevation being most common. Sweat chloride test with chloride ion concentrations ≥ 60 mmol per liter is characteristic of cystic fibrosis.[50]

CF may present with neonatal cholestasis, which histologically shows ductular proliferation and neutrophilic infiltration in portal tracts. Giant cell transformation of hepatocytes and extramedullary hemopoiesis may be observed. Steatosis is a common finding in the liver in CF, affecting between one-third and two-third of patients. Steatosis in CF is not only seen in malnourished patients but also in patients on appropriate supplemental diets. Histologically, there is azonal distribution of micro and macrovesicular fat. Focal biliary cirrhosis is a pathognomic feature of CF. The liver is not grossly or diffusely nodular but may show scattered gray white scars. Microscopically, the affected areas show fibrous portal expansion with portal-portal and portal-central bridging fibrous septa (Fig. 6A). Long-standing CF may lead to multilobar cirrhosis, which leads to a profoundly lobulated liver, in which large lobules are separated by deep clefts formed by broad bands of fibrous tissue. In all instances, the interlobular bile ducts and ductules at the periphery of the portal tracts may contain an inspissated eosinophilic material (Fig. 6B), which is PAS positive and resistant to digestion with diastase. Cholangitis is a common and recurrent complication of CF, which may be seen histologically as a neutrophilic infiltrate around and within bile ducts, with or without rupture of ducts containing the inspissated eosinophilic material.

α-1 ANTITRYPSIN DEFICIENCY

Alpha-1 antitrypsin deficiency (AATD) is one of the most common genetic disorders in the general population. It is caused by mutations in *SERPINA1*, located on chromosome 14, which encodes for α-1 antitrypsin (AAT) protein. AAT is a large 394 amino acid, 52 kDa glycoprotein and serine protease inhibitor that protects tissues, mainly liver and lungs, from the proteolytic action of enzymes, particularly neutrophil elastase.[51]

Over 150 mutations have been described in the *SERPINA1* gene.[54] A single amino acid substitution caused by point mutations leads to mutated variants, which are classified based on their mobility on an isoelectric gradient when compared with the normal M allele. Normal α-1 antitrypsin migrates in the middle (M) and variants are designated A–L if they migrate faster than M, or N–Z if they migrate more slowly. The most commonly encountered abnormal alleles in clinical practice are the Z allele (Glu342Lys) followed by the S allele (Glu264Val).[54,68] These alleles result in a protein that cannot be folded normally within the endoplasmic reticulum, and consequently cannot be secreted into blood, leading to low serum levels of AAT. The abnormal protein polymerizes and accumulates within hepatocytes causing activation of the unfolded protein response (UPR). The latter is a cellular stress response that induces chaperones to assist with

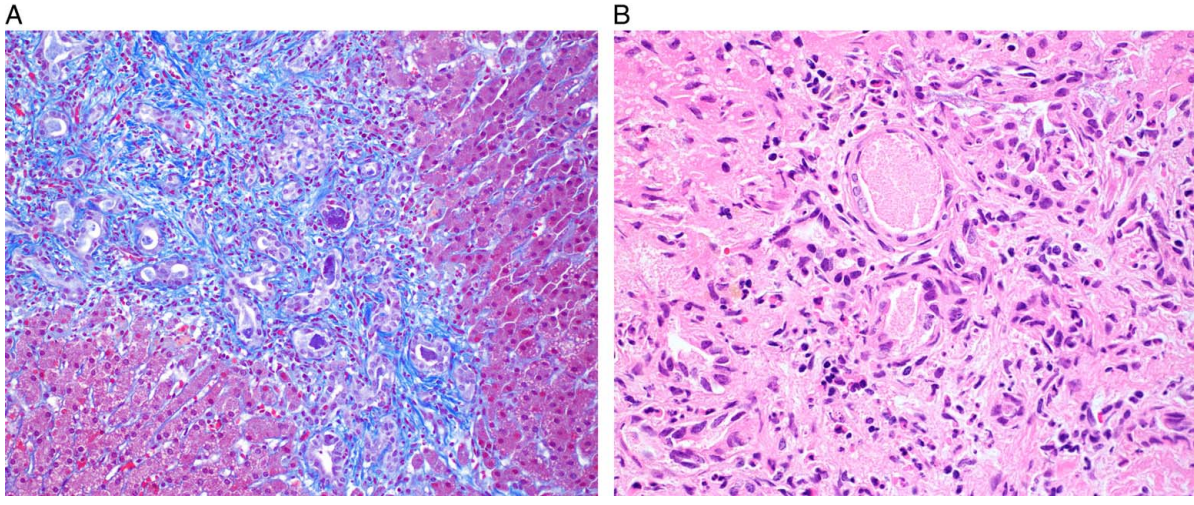

FIGURE 6. A, Trichrome stain showing focal biliary cirrhosis characterized by an expanded fibrotic portal tract containing numerous ducts, which contain a thick proteinaceous material that stains magenta on the trichrome stain . B, Cystic fibrosis often shows dilated bile ducts with a pink proteinaceous material. Ductular proliferation and a mild mixed inflammatory infiltrate are also noted (A,B). Courtesy of Dr. Hanlin Wang, University of California, Los Angeles. Please see this image in color online.

degradation of misfolded proteins, which in turn leads to apoptosis of cells containing the misfolded proteins.[60,69]

The PiZZ genotype causes clinically severe liver disease leading to liver fibrosis and risk of developing hepatocellular carcinoma.[68,69] Heterozygosity for the Z or S alleles (PiMZ and PiMS) is linked to the development of lung and liver disease in patients with other risk factors such as smoking and alcohol use. Studies have shown that the diagnosis of AATD may be missed in patients who have other comorbidities, particularly steatotic liver disease, whether associated with alcohol or metabolic dysfunction.[70] Although AAT is mostly diagnosed in adults, it can present in infancy as neonatal cholestasis.

Microscopically, the abnormal protein accumulates within hepatocytes as variably sized eosinophilic intracytoplasmic globules, which may be surrounded by a clear halo (Fig. 7A). The accumulation begins in periportal hepatocytes (acinar zone 1) and progressively extends into zones 2 and 3. The intracytoplasmic globules are PAS positive and resistant to digestion with diastase (Fig. 7B). Immunohistochemical stain for AAT highlights these globules; the larger globules usually have a dark peripheral ring with a light brown center (Fig. 7C). Both PASD and immunohistochemical stains highlight smaller granules in addition to the larger well-defined globules. Varying degrees of lymphocytic inflammation and ductular reaction are present in the portal tracts. As the disease advances, portal fibrosis progresses to bridging fibrosis and cirrhosis. Neonatal cholestasis due to AATD shows features of neonatal hepatitis, such as cellular and canalicular cholestasis, multinucleated giant hepatocytes with variable inflammation, and extramedullary hematopoiesis. However, intracytoplasmic globules are not visible in early life, as the protein has not had enough time to accumulate in substantial amounts. Some cases of neonatal cholestasis may show paucity of intrahepatic bile ducts and others may show a mild ductular reaction.

DRUG-INDUCED LIVER INJURY

The liver plays a vital role in metabolism of drugs and xenobiotics; uptake into hepatocytes and elimination into the bloodstream or bile require transporters across the basolateral and apical membranes of hepatocytes, respectively. Uptake of drugs at the basolateral membrane is facilitated by organic anion transporting peptides (OATPs), organic anion transporters (OATs) and organic cation transporters (OCTs). Efflux transporters on the basolateral membrane include multidrug resistance-associated proteins MRP3 and MRP4, and those on the canalicular membrane include BSEP, MDR3, MRP2, and ATP8B1.[71] Thus, mutations in any of these transporters lead to impaired metabolism and/or elimination of drugs and xenobiotics. Furthermore, a large number of drugs and xenobiotics have

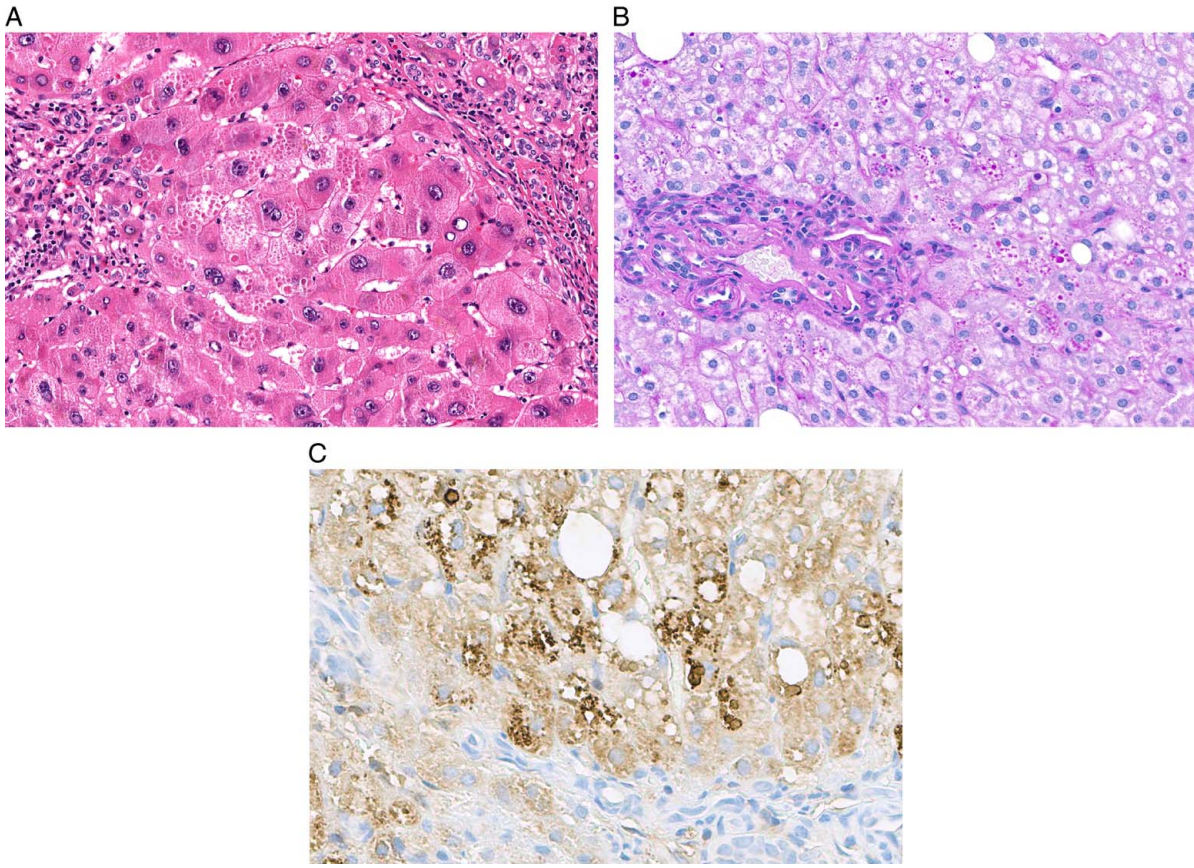

FIGURE 7. A, The abnormal protein in α-1 antitrypsin deficiency accumulates within periportal hepatocytes as eosinophilic intracytoplasmic globules surrounded by a halo. B, The globules are positive with PAS stain and resist digestion with diastase. C, Immunohistochemical stain for α-1 antitrypsin highlights the globules as well as smaller granules within hepatocytes. The large globules have a dark peripheral ring and pale center. Please see this image in color online.

a transcriptional or functional inhibitory effect on these transporters, setting the stage for drug-induced cholestasis.[72–74]

Drug-induced cholestasis can present as an acute illness with or without jaundice. The symptoms may be nonspecific such as nausea, anorexia, and fatigue. Symptoms may occur after weeks or sometimes months after the initiation of the offending agent. Liver enzymes, including ALT, ALP, and total bilirubin are generally elevated in serum. Diagnosing drug-induced cholestasis can be challenging and a detailed clinical history is needed to exclude competing causes of cholestasis. All of the medications consumed by the patients in the last 12 months should be evaluated.[75] Temporal association between initiation of the medication and onset of the symptoms provides vital evidence for establishing the diagnosis.

MANAGEMENT

Management of ALGS and PFIC focuses on improving cholestasis and relieving pruritus, which is often debilitating, as well as providing nutritional supplementation to correct fat malabsorption and related vitamin deficiencies. In addition, surveillance for malignancy is required in patients with PFIC-2 and PFIC-4 due to the unusually high risk for development of malignancy.

Ursodeoxycholic acid (UDCA), rifampin, and cholestyramine are the mainstays for treatment of pruritus. UDCA is a hydrophilic bile acid that is hypothesized to induce the expression of BSEP and MDR3, thereby improving symptoms.[30] In more recent years, intestinal bile acid transporter (IBAT) inhibitors have expanded the armamentarium for treating cholestasis and pruritus that are refractory to standard medications.[76] IBATs inhibit intestinal absorption of bile salts, thus reducing the amount of bile salts delivered to the liver via enterohepatic circulation. A newer therapy involving a BSEP-enhancer molecule, such as 4-phenylbutyrate, has demonstrated improvement in liver function and pruritus.[77] Similarly, a CFTR/ABCC7 potentiator has been demonstrated to cause functional rescue of missense mutations in the highly conserved ABC transporter motifs of *ABCB11* and *ABCB4*. These are promising developments, as they offer the potential for targeted therapies to address the underlying genetic causes of these disorders.[78] When medical management fails, surgical intervention may be necessary. The main goal is biliary diversion to interrupt the circulation of bile acids; strategies include partial external biliary diversion (PEBD), partial internal biliary diversion (PIBD), and ileal exclusion (IE). These procedures have varied responses and complications in individual patients.[43,79]

Liver transplantation (LT) offers a viable therapeutic option for intractable symptoms, particularly pruritus as well as for end-stage liver disease. LT abrogates the genetic abnormality in the liver but long-term prognosis is determined by extrahepatic manifestations and complications that result from the altered milieu following liver transplantation. In ALGS, liver function improves in 90% of patients, and 5-year survival rates are favorable, but may be limited by cardiac and renal manifestations of ALGS.[21] LT does not protect PFIC-1 patients from extrahepatic manifestations of the disease, which continue to progress. Diarrhea may worsen because the normal load of bile salts now processed by the allograft overwhelms the FIC-1 deficient small intestine. The allograft is also prone to steatotic liver disease; the exact pathogenesis if uncertain, but is undoubtedly related to altered metabolic handling of lipids. Approximately 8% of PFIC-2 patients develop recurrent disease following LT due to development of alloreactive antibodies to BSEP. Aggressive immunosuppressive therapy is often required to treat recurrent disease.[80] Steatosis has been reported in allografts of patients transplanted for PFIC-5. Combined bowel-liver transplant is favored in patients with PFIC-6 to prevent post-transplant cholestasis.

UDCA and antipruritic medications form the mainstay of management of BRIC and ICP. A combination of UDCA and rifampicin has been used in nonresponsive cases of BRIC.[81] Newer therapies including fibrates, such as fenofibrate (ileal bile acid transport inhibitor) can also be used to manage the pruritus of BRIC.[70] UDCA is the treatment of choice for ICP and has not been shown to have adverse effects on the fetus.[82]

Nutritional supplementation and prevention of complications constitute a large component of CF management. UDCA is widely used to halt or stop progression of fibrosis.[67] CFTR correctors and potentiators aim to prevent or stop the progression of liver disease by correcting and potentiating CFTR in cholangiocytes, endothelial cells, and platelets.[83–85] Additional strategies involve inhibition of Src tyrosine kinases targeting the TLR4-mediated inflammatory process and use of FXR agonists, FGF19 analogs, and other fibroblast growth factors[85–87] to ease progression of cholestasis.

Following diagnosis of AATD, patients are monitored for development and progression of liver fibrosis and development of hepatocellular carcinoma. Emerging medical therapies currently in clinical trials include carbamazepine, which enhance autophagy and clearance of the mutant Z protein.[60,88] RNA-interference (RNAi) offers another promising therapeutic strategy; iRNAs abrogate cellular production of the abnormal protein[89,90] and halt progression of liver damage and fibrosis. Liver transplantation is a viable therapeutic option for patients with end-stage liver disease due to AATD[91]; the procedure ameliorates the genetic deficiency.

In the majority of cases of DILI, the symptoms are alleviated after withdrawal of the offending medication and do not lead to chronic liver disease. However, studies have shown that persistent use of the medication for >6 months has been associated with the development of chronic liver disease and advanced fibrosis. In addition to the withdrawal of the offending agent, treatment of symptoms such as pruritus is important.[92,93]

CONCLUSIONS

Molecular advancements over the last few decades have unraveled multiple genetic defects as the underlying causes of previously "idiopathic" cholestatic liver diseases. The mutated genes often play a critical role in transmembrane transport of molecules, formation of components of bile or regulation of feedback loops in bile formation. Disruption of any of these processes leads to varying degrees of cholestasis and liver damage. Looking ahead, next-generation sequencing is poised to play an increasingly pivotal role in the early detection of disease-causing genetic variants and the discovery of new genetic causes of cholestasis. In addition, the surge in innovative therapeutic strategies for cholestatic conditions, driven by our growing

understanding of their molecular pathophysiology, offers the prospect of new precision treatments and potential cures for these complex disorders. In short, researchers and clinicians now have powerful tools to uncover the genetic underpinnings of challenging cholestatic liver diseases, promising improved diagnosis, evolution of targeted therapies, and better outcomes for patients.

REFERENCES

1. Matte U, Mourya R, Miethke A, et al. Analysis of gene mutations in children with cholestasis of undefined etiology. *J Pediatr Gastroenterol Nutr*. 2010;51:488–493.
2. Colombo C, Okolicsanyi L, Strazzabosco M. Advances in familial and congenital cholestatic diseases. Clinical and diagnostic implications. *Dig Liver Dis*. 2000;32:152–159.
3. Carlton VE, Pawlikowska L, Bull LN. Molecular basis of intrahepatic cholestasis. *Ann Med*. 2004;36:606–617.
4. Goldschmidt ML, Mourya R, Connor J, et al. Increased frequency of double and triple heterozygous gene variants in children with intrahepatic cholestasis. *Hepatol Res*. 2016;46: 306–311.
5. Lipinski P, Ciara E, Jurkiewicz D, et al. Targeted next-generation sequencing in diagnostic approach to monogenic cholestatic liver disorders-single-center experience. *Front Pediatr*. 2020;8:414.
6. Nicastro E, D'Antiga L. Next generation sequencing in pediatric hepatology and liver transplantation. *Liver Transpl*. 2018;24:282–293.
7. Almes M, Spraul A, Ruiz M, et al. Targeted-capture next-generation sequencing in diagnosis approach of pediatric cholestasis. *Diagnostics (Basel)*. 2022;12:1169.
8. Feldman AG, Sokol RJ. Neonatal cholestasis: emerging molecular diagnostics and potential novel therapeutics. *Nat Rev Gastroenterol Hepatol*. 2019;16:346–360.
9. Chen HL, Wu SH, Hsu SH, et al. Jaundice revisited: recent advances in the diagnosis and treatment of inherited cholestatic liver diseases. *J Biomed Sci*. 2018;25:75.
10. Klomp LW, Bull LN, Knisely AS, et al. A missense mutation in FIC1 is associated with greenland familial cholestasis. *Hepatology*. 2000;32:1337–1341.
11. Amirneni S, Haep N, Gad MA, et al. Molecular overview of progressive familial intrahepatic cholestasis. *World J Gastroenterol*. 2020;26:7470–7484.
12. Stapelbroek JM, Peters TA, van Beurden DH, et al. ATP8B1 is essential for maintaining normal hearing. *Proc Natl Acad Sci U S A*. 2009;106:9709–9714.
13. Kamath BM, Bason L, Piccoli DA, et al. Consequences of JAG1 mutations. *J Med Genet*. 2003;40:891–895.
14. Krantz ID, Piccoli DA, Spinner NB. Clinical and molecular genetics of Alagille syndrome. *Curr Opin Pediatr*. 1999;11: 558–564.
15. Li L, Krantz ID, Deng Y, et al. Alagille syndrome is caused by mutations in human Jagged1, which encodes a ligand for Notch1. *Nat Genet*. 1997;16:243–251.
16. Penton AL, Leonard LD, Spinner NB. Notch signaling in human development and disease. *Semin Cell Dev Biol*. 2012;23: 450–457.
17. Turnpenny PD, Ellard S. Alagille syndrome: pathogenesis, diagnosis and management. *Eur J Hum Genet*. 2012;20: 251–257.
18. Semenova N, Kamenets E, Annenkova E, et al. Clinical characterization of alagille syndrome in patients with cholestatic liver disease. *Int J Mol Sci*. 2023;24:11758.
19. Ayoub MD, Kamath BM. Alagille syndrome: diagnostic challenges and advances in management. *Diagnostics (Basel)*. 2020;10:907.
20. Deprettere A, Portmann B, Mowat AP. Syndromic paucity of the intrahepatic bile ducts: diagnostic difficulty; severe morbidity throughout early childhood. *J Pediatr Gastroenterol Nutr*. 1987;6:865–871.
21. Vandriel SM, Li LT, She H, et al. Natural history of liver disease in a large international cohort of children with Alagille syndrome: Results from the GALA study. *Hepatology*. 2023;77: 512–529.
22. Vinayagamoorthy V, Srivastava A, Sarma MS. Newer variants of progressive familial intrahepatic cholestasis. *World J Hepatol*. 2021;13:2024–2038.
23. Baker A, Kerkar N, Todorova L, et al. Systematic review of progressive familial intrahepatic cholestasis. *Clin Res Hepatol Gastroenterol*. 2019;43:20–36.
24. Baker A, Kerkar N, Todorova L, et al. Erratum to "Systematic review of progressive familial intrahepatic cholestasis" [Clin. Res. Hepatol. Gastroenterol. 43 (2019) 20-36]. *Clin Res Hepatol Gastroenterol*. 2020;44:115.
25. Hassan S, Hertel P. Overview of progressive familial intrahepatic cholestasis. *Clin Liver Dis*. 2022;26:371–390.
26. Clayton RJ, Iber FL, Ruebner BH, et al. Byler disease. Fatal familial intrahepatic cholestasis in an Amish kindred. *Am J Dis Child*. 1969;117:112–124.
27. Ray NB, Durairaj L, Chen BB, et al. Dynamic regulation of cardiolipin by the lipid pump Atp8b1 determines the severity of lung injury in experimental pneumonia. *Nat Med*. 2010;16: 1120–1127.
28. van der Woerd WL, van Haaften-Visser DY, van de Graaf SF, et al. Mutational analysis of ATP8B1 in patients with chronic pancreatitis. *PLoS One*. 2013;8:e80553.
29. Alvarez L, Jara P, Sanchez-Sabate E, et al. Reduced hepatic expression of farnesoid X receptor in hereditary cholestasis associated to mutation in ATP8B1. *Hum Mol Genet*. 2004;13: 2451–2460.
30. Morotti RA, Suchy FJ, Magid MS. Progressive familial intrahepatic cholestasis (PFIC) type 1, 2, and 3: a review of the liver pathology findings. *Semin Liver Dis*. 2011;31:3–10.
31. van Mil SW, van der Woerd WL, van der Brugge G, et al. Benign recurrent intrahepatic cholestasis type 2 is caused by mutations in ABCB11. *Gastroenterology*. 2004;127:379–384.
32. Strautnieks SS, Byrne JA, Pawlikowska L, et al. Severe bile salt export pump deficiency: 82 different ABCB11 mutations in 109 families. *Gastroenterology*. 2008;134:1203–1214.
33. Knisely AS, Strautnieks SS, Meier Y, et al. Hepatocellular carcinoma in ten children under five years of age with bile salt export pump deficiency. *Hepatology*. 2006;44:478–486.
34. Pawlikowska L, Strautnieks S, Jankowska I, et al. Differences in presentation and progression between severe FIC1 and BSEP deficiencies. *J Hepatol*. 2010;53:170–178.
35. Scheimann AO, Strautnieks SS, Knisely AS, et al. Mutations in bile salt export pump (ABCB11) in two children with progressive familial intrahepatic cholestasis and cholangiocarcinoma. *J Pediatr*. 2007;150:556–559.
36. Evason K, Bove KE, Finegold MJ, et al. Morphologic findings in progressive familial intrahepatic cholestasis 2 (PFIC2): correlation with genetic and immunohistochemical studies. *Am J Surg Pathol*. 2011;35:687–696.
37. Degiorgio D, Colombo C, Seia M, et al. Molecular characterization and structural implications of 25 new ABCB4 mutations in progressive familial intrahepatic cholestasis type 3 (PFIC3). *Eur J Hum Genet*. 2007;15:1230–1238.
38. Davit-Spraul A, Gonzales E, Baussan C, et al. The spectrum of liver diseases related to ABCB4 gene mutations: pathophysiology and clinical aspects. *Semin Liver Dis*. 2010;30:134–146.
39. Vij M, Safwan M, Shanmugam NP, et al. Liver pathology in severe multidrug resistant 3 protein deficiency: a series of 10 pediatric cases. *Ann Diagn Pathol*. 2015;19:277–282.
40. Gumbiner B, Lowenkopf T, Apatira D. Identification of a 160-kDa polypeptide that binds to the tight junction protein ZO-1. *Proc Natl Acad Sci U S A*. 1991;88:3460–3464.
41. Sambrotta M, Thompson RJ. Mutations in TJP2, encoding zona occludens 2, and liver disease. *Tissue Barriers*. 2015;3: e1026537.
42. Sambrotta M, Strautnieks S, Papouli E, et al. Mutations in TJP2 cause progressive cholestatic liver disease. *Nat Genet*. 2014;46:326–328.

43. Furuse M, Fujita K, Hiiragi T, et al. Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. *J Cell Biol*. 1998;141:1539–1550.
44. Vij M, Shanmugam NP, Reddy MS, et al. Paediatric hepatocellular carcinoma in tight junction protein 2 (TJP2) deficiency. *Virchows Arch*. 2017;471:679–683.
45. Himes RW, Mojarrad M, Eslahi A, et al. NR1H4-related progressive familial intrahepatic cholestasis 5: further evidence for rapidly progressive liver failure. *J Pediatr Gastroenterol Nutr*. 2020;70:e111–e113.
46. Forman BM, Goode E, Chen J, et al. Identification of a nuclear receptor that is activated by farnesol metabolites. *Cell*. 1995;81: 687–693.
47. Aldrian D, Vogel GF, Frey TK, et al. Congenital diarrhea and cholestatic liver disease: phenotypic spectrum associated with MYO5B mutations. *J Clin Med*. 2021;10:481.
48. 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;60:301–310.
49. Gonzales E, Taylor SA, Davit-Spraul A, et al. MYO5B mutations cause cholestasis with normal serum gamma-glutamyl transferase activity in children without microvillous inclusion disease. *Hepatology*. 2017;65:164–173.
50. Stonebraker JR, Ooi CY, Pace RG, et al. Features of severe liver disease with portal hypertension in patients with cystic fibrosis. *Clin Gastroenterol Hepatol*. 2016;14:1207–15.e3.
51. Fromme M, Schneider CV, Trautwein C, et al. Alpha-1 antitrypsin deficiency: a re-surfacing adult liver disorder. *J Hepatol*. 2022;76:946–958.
52. Halawi A, Ibrahim N, Bitar R. Triggers of benign recurrent intrahepatic cholestasis and its pathophysiology: a review of literature. *Acta Gastroenterol Belg*. 2021;84:477–486.
53. Strubbe B, Geerts A, Van Vlierberghe H, et al. Progressive familial intrahepatic cholestasis and benign recurrent intrahepatic cholestasis: a review. *Acta Gastroenterol Belg*. 2012;75: 405–410.
54. Santos G, Turner AM. Alpha-1 antitrypsin deficiency: an update on clinical aspects of diagnosis and management. *Fac Rev*. 2020;9:1.
55. Luketic VA, Shiffman ML. Benign recurrent intrahepatic cholestasis. *Clin Liver Dis*. 2004;8:133–149, vii.
56. Dixon PH, Williamson C. The molecular genetics of intrahepatic cholestasis of pregnancy. *Obstet Med*. 2008;1:65–71.
57. Dixon PH, Williamson C. The pathophysiology of intrahepatic cholestasis of pregnancy. *Clin Res Hepatol Gastroenterol*. 2016; 40:141–153.
58. Pauli-Magnus C, Lang T, Meier Y, et al. Sequence analysis of bile salt export pump (ABCB11) and multidrug resistance p-glycoprotein 3 (ABCB4, MDR3) in patients with intrahepatic cholestasis of pregnancy. *Pharmacogenetics*. 2004;14:91–102.
59. Paus TC, Schneider G, Van De Vondel P, et al. Diagnosis and therapy of intrahepatic cholestasis of pregnancy. *Z Gastroenterol*. 2004;42:623–628.
60. Kandregula CAB, Smilin Bell Aseervatham G, Bentley GT, et al. Alpha-1 antitrypsin: associated diseases and therapeutic uses. *Clin Chim Acta*. 2016;459:109–116.
61. Ambros-Rudolph CM, Glatz M, Trauner M, et al. The importance of serum bile acid level analysis and treatment with ursodeoxycholic acid in intrahepatic cholestasis of pregnancy: a case series from central Europe. *Arch Dermatol*. 2007;143: 757–762.
62. Bicocca MJ, Sperling JD, Chauhan SP. Intrahepatic cholestasis of pregnancy: review of six national and regional guidelines. *Eur J Obstet Gynecol Reprod Biol*. 2018;231:180–187.
63. Shteinberg M, Haq IJ, Polineni D, et al. Cystic fibrosis. *Lancet*. 2021;397:2195–2211.
64. Zaibak F, Williamson R. Genetics and genomics: providing new opportunities for the translation of basic research to clinical care for cystic fibrosis. *Expert Rev Respir Med*. 2008;2:1–5.
65. Lukacs GL, Verkman AS. CFTR: folding, misfolding and correcting the DeltaF508 conformational defect. *Trends Mol Med*. 2012;18:81–91.
66. Sharma N, Cutting GR. The genetics and genomics of cystic fibrosis. *J Cyst Fibros*. 2020;19 Suppl 1:S5–S9.
67. Dana J, Debray D, Beaufrere A, et al. Cystic fibrosis-related liver disease: clinical presentations, diagnostic and monitoring approaches in the era of CFTR modulator therapies. *J Hepatol*. 2022;76:420–434.
68. Narayanan P, Mistry PK. Update on alpha-1 antitrypsin deficiency in liver disease. *Clin Liver Dis (Hoboken)*. 2020;15: 228–235.
69. Crowther DC, Belorgey D, Miranda E, et al. Practical genetics: alpha-1-antitrypsin deficiency and the serpinopathies. *Eur J Hum Genet*. 2004;12:167–172.
70. European Association for the Study of the L. EASL Clinical Practice Guidelines on genetic cholestatic liver diseases. *J Hepatol*. 2024;81:303–325.
71. Jetter A, Kullak-Ublick GA. Drugs and hepatic transporters: a review. *Pharmacol Res*. 2020;154:104234.
72. Yang K, Kock K, Sedykh A, et al. An updated review on drug-induced cholestasis: mechanisms and investigation of physicochemical properties and pharmacokinetic parameters. *J Pharm Sci*. 2013;102:3037–3057.
73. Kolaric TO, Nincevic V, Smolic R, et al. Mechanisms of hepatic cholestatic drug injury. *J Clin Transl Hepatol*. 2019;7: 86–92.
74. Vitale G, Mattiaccio A, Conti A, et al. Molecular and clinical links between drug-induced cholestasis and familial intrahepatic cholestasis. *Int J Mol Sci*. 2023;24:5283.
75. Singh KA, Kumar SE, Zachariah UG, et al. Single-centre experience with low-volume plasma exchange and low-dose steroid to treat patients with idiosyncratic drug-induced acute liver failure. *J Clin Exp Hepatol*. 2024;14:101303.
76. Kamath BM, Stein P, Houwen RHJ, et al. Potential of ileal bile acid transporter inhibition as a therapeutic target in Alagille syndrome and progressive familial intrahepatic cholestasis. *Liver Int*. 2020;40:1812–1822.
77. Naoi S, Hayashi H, Inoue T, et al. Improved liver function and relieved pruritus after 4-phenylbutyrate therapy in a patient with progressive familial intrahepatic cholestasis type 2. *J Pediatr*. 2014;164:1219–27.e3.
78. Delaunay JL, Bruneau A, Hoffmann B, et al. Functional defect of variants in the adenosine triphosphate-binding sites of ABCB4 and their rescue by the cystic fibrosis transmembrane conductance regulator potentiator, ivacaftor (VX-770). *Hepatology*. 2017;65:560–570.
79. Whitington PF, Whitington GL. Partial external diversion of bile for the treatment of intractable pruritus associated with intrahepatic cholestasis. *Gastroenterology*. 1988;95:130–136.
80. Jara P, Hierro L, Martinez-Fernandez P, et al. Recurrence of bile salt export pump deficiency after liver transplantation. *N Engl J Med*. 2009;361:1359–1367.
81. Helgadottir H, Folvik G, Vesterhus M. Improvement of cholestatic episodes in patients with benign recurrent intrahepatic cholestasis (BRIC) treated with rifampicin. A long-term follow-up. *Scand J Gastroenterol*. 2023;58: 512–520.
82. Ovadia C, Sajous J, Seed PT, et al. Ursodeoxycholic acid in intrahepatic cholestasis of pregnancy: a systematic review and individual participant data meta-analysis. *Lancet Gastroenterol Hepatol*. 2021;6:547–558.
83. Heijerman HGM, McKone EF, Downey DG, et al. Efficacy and safety of the elexacaftor plus tezacaftor plus ivacaftor combination regimen in people with cystic fibrosis homozygous for the F508del mutation: a double-blind, randomised, phase 3 trial. *Lancet*. 2019;394:1940–1948.
84. Wainwright CE, Elborn JS, Ramsey BW. Lumacaftor-ivacaftor in patients with cystic fibrosis homozygous for Phe508del CFTR. *N Engl J Med*. 2015;373:1783–1784.
85. Fiorotto R, Amenduni M, Mariotti V, et al. Src kinase inhibition reduces inflammatory and cytoskeletal changes in DeltaF508 human cholangiocytes and improves cystic fibrosis transmembrane conductance regulator correctors efficacy. *Hepatology*. 2018;67:972–988.

86. Hirschfield GM, Chazouilleres O, Drenth JP, et al. Effect of NGM282, an FGF19 analogue, in primary sclerosing cholangitis: a multicenter, randomized, double-blind, placebo-controlled phase II trial. *J Hepatol*. 2019;70:483–493.
87. Lin H, Zhou C, Hou Y, et al. Paracrine fibroblast growth factor 1 functions as potent therapeutic agent for intrahepatic cholestasis by downregulating synthesis of bile Acid. *Front Pharmacol*. 2019;10:1515.
88. Teckman JH. Emerging concepts and human trials in alpha-1-antitrypsin deficiency liver disease. *Semin Liver Dis*. 2017;37:152–158.
89. Clark VC, Strange C, Strnad P, et al. Fazirsiran for adults with alpha-1 antitrypsin deficiency liver disease: a phase 2 placebo controlled trial (SEQUOIA). *Gastroenterology*. 2024;167:1008–18.e5.
90. Strnad P, Mandorfer M, Choudhury G, et al. Fazirsiran for liver disease associated with alpha(1)-antitrypsin deficiency. *N Engl J Med*. 2022;387:514–524.
91. Torres-Duran M, Lopez-Campos JL, Barrecheguren M, et al. Alpha-1 antitrypsin deficiency: outstanding questions and future directions. *Orphanet J Rare Dis*. 2018;13:114.
92. Sundaram V, Bjornsson ES. Drug-induced cholestasis. *Hepatol Commun*. 2017;1:726–735.
93. Bjornsson ES, Devarbhavi HC. Drug-induced cholestatic liver diseases. *Hepatology*. 2024. doi: 10.1097/HEP.0000000000001052. Online ahead of print.