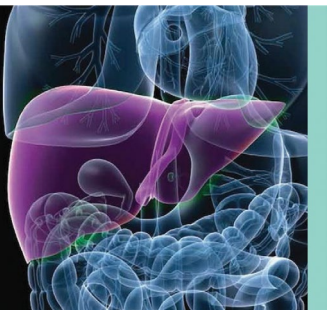

Genetic Disorders of Bile Acid Transport

Anne S. Henkel, M.D.

Cholestasis, the slowing or stoppage of bile flow, causes toxic bile acids to accumulate in the liver resulting in liver injury. Impaired bile flow can result from a variety of conditions, the most common of which are obstruction of the extrahepatic bile ducts (e.g., gallstones, tumors, or strictures related to primary sclerosing cholangitis), drug toxicity, or primary biliary cholangitis. Although these conditions have clear genetic influence, they are multifactorial in etiology. In addition, there is a collection of relatively rare conditions with monogenic causes manifesting as intrahepatic cholestasis. This review focuses on these genetic disorders of bile acid transport.

BILE ACID SYNTHESIS AND TRANSPORT

Under normal physiological conditions, bile acids are shuttled between the liver and small intestine through a process termed the enterohepatic circulation (Fig. 1). Although the kinetics of the enterohepatic circulation have been known for nearly a century, the molecular mechanisms facilitating the proper flow of bile acids have been elucidated largely over the past three decades.[1]

Bile acids are synthesized in hepatocytes and actively secreted across the canalicular membrane into small terminal bile ducts, termed bile canaliculi. The major canalicular bile acid transporter is the bile salt export pump (BSEP) encoded by *ABCB11* (Fig. 2). Other components of bile, including phospholipids and cholesterol, are exported with bile acids across the canalicular membrane via specific transporters as shown in Fig. 2. Proper bile flow requires the coordinated action of all of the canalicular membrane transporters, and disruption of any one transporter can lead to cholestasis.

After bile acids reach the small intestine and have performed their critical function in lipid solubilization, they

Abbreviations: ALGS, Alagille syndrome; BRIC, benign recurrent intrahepatic cholestasis; BSEP, bile salt export pump; CBAS, congenital bile acid synthesis; CYP7A1, cholesterol 7α hydroxylase; FGF19, fibroblast growth factor 19; FIC1, familial intrahepatic cholestasis type 1; FXR, farnesoid X receptor; GGT, gamma glutamyl transferase; ICP, intrahepatic cholestasis of pregnancy; MDR3, multidrug resistance protein 3; MVID, microvillus inclusion disease; MYO5B, Myosin 5B; NTCP, sodium-dependent taurocholate cotransporting peptide; PFIC, progressive familial intrahepatic cholestasis; SHP, short heterodimer partner; TJP, tight junction protein.

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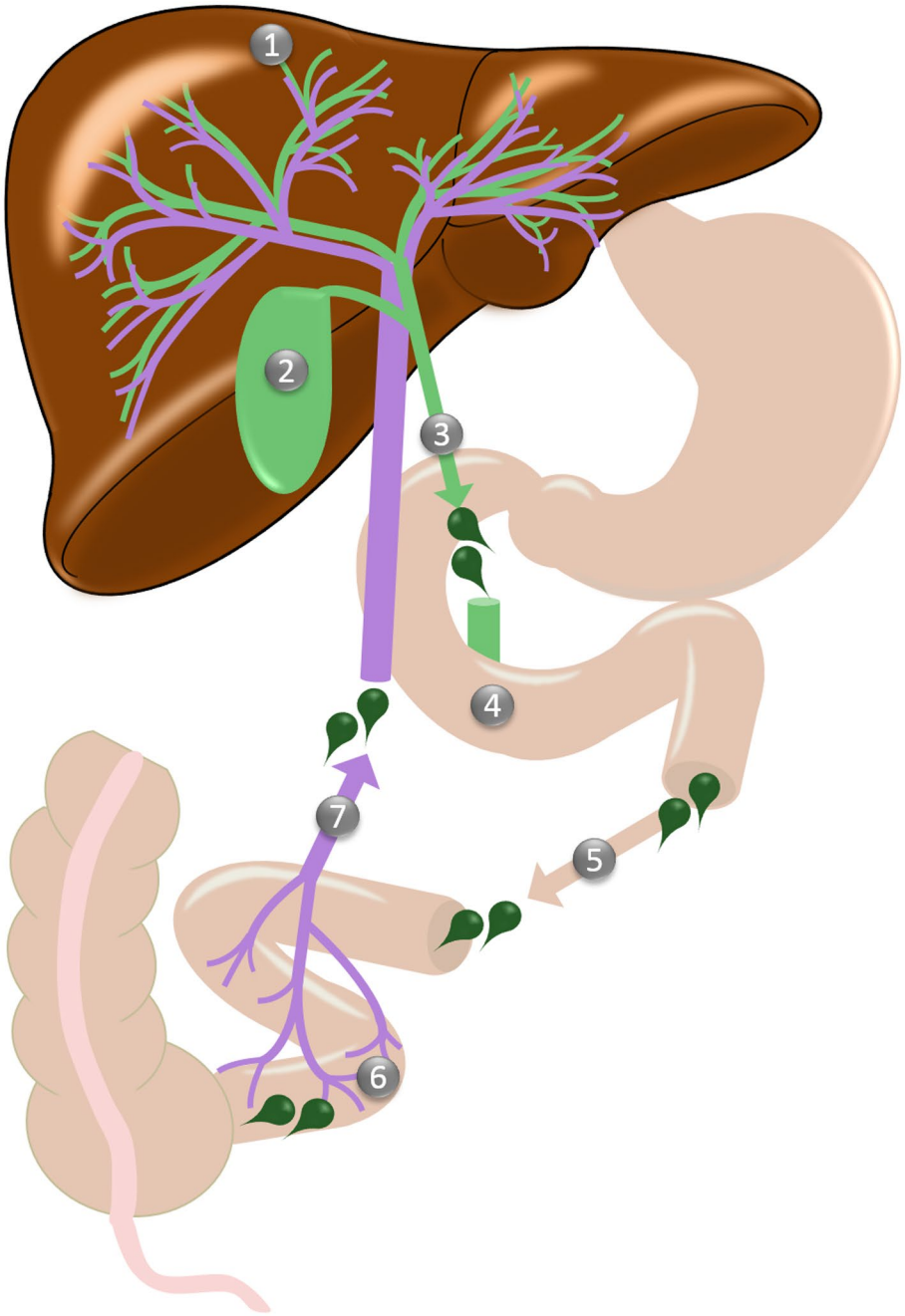

FIG 1 The enterohepatic circulation of bile acids. Bile acids are synthesized in the liver (1), stored in the gallbladder (2), and transported through the biliary tree (3) to the duodenum (4), where they aid in the solubilization and transport of dietary lipids. Bile acids travel through the entire small intestine (5) to the ileum, where they are reabsorbed (6) and returned to the liver via the portal circulation (7).

are actively taken up in the ileum and returned to the liver via the portal circulation (Figs. 1 and 2). In each cycle of the enterohepatic circulation, about 5% of bile acids escape reabsorption in the ileum, and the liver must synthesize new bile acids to replace those that are lost. Bile acids are synthesized from cholesterol through a series of enzymatic steps, many of which are subject to genetic disruption[1] (Table 1). Bile acids regulate their own synthesis through a feedback loop involving activation of the endogenous bile acid receptor, the farnesoid X receptor (FXR), encoded by *NR1H4*.[2] In addition to regulating bile acid synthesis, FXR regulates several other transporters that are critical to the normal flux of bile acids through the hepatocyte (Fig. 2).

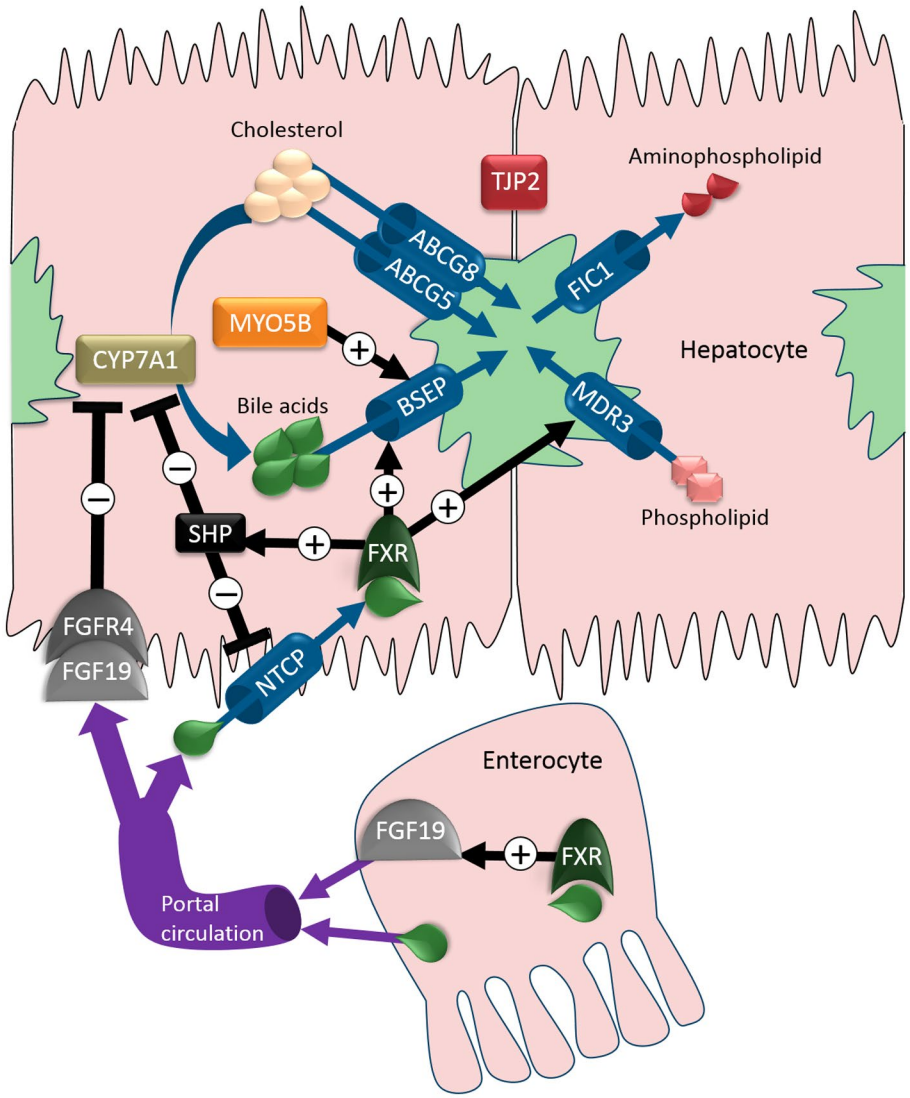

FIG 2 Molecular mechanisms of bile acid synthesis and transport. Bile acids are synthesized in hepatocytes from cholesterol through a series of enzymatic steps controlled by the rate-limiting cytochrome P450 enzyme, CYP7A1. Bile acids are exported from the hepatocyte into bile canaliculi by BSEP encoded by *ABCB11*. The major canalicular phospholipid transporter is the MDR3 encoded by *ABCB4*. Biliary cholesterol secretion is mediated by the ABCG5/8 heterodimer. FIC1, encoded by *ATP8B1*, is a canalicular membrane aminophospholipid flippase. After participating in lipid solubilization in the proximal small intestine, bile acids are actively taken up by enterocytes of the ileum and returned to the liver via the portal circulation. Hepatic uptake of bile acids is controlled primarily by the basolateral membrane transporter, NTCP. Bile salts regulate their own synthesis via two distinct mechanisms of feedback inhibition of *CYP7A1*. Within hepatocytes, bile acids bind to their endogenous receptor, the FXR, inducing transcription of the SHP, which potently represses transcription of *CYP7A1*. Feedback regulation of *CYP7A1* also originates from the gut via a hormonal signal. Bile salts taken up in the ileum bind FXR in the enterocyte stimulating transcription of FGF19. FGF19 is released into the portal circulation and taken up in the liver, where it binds its receptor FGFR4 to negatively regulate *CYP7A1*. Activated FXR also induces BSEP and MDR3 to promote the export of bile salts and phospholipids from hepatocytes into bile canaliculi. In addition, through activation of SHP, FXR suppresses NTCP, thereby reducing bile salt entry into the hepatocyte. TJP2 functions to prevent leakage of bile acids into hepatocytes. MYO5B regulates intracellular protein trafficking, including localization of BSEP to the canalicular membrane.

In summary, bile acid synthesis and transport are tightly regulated processes with multiple steps that are prone to disruption. Genetic mutations have been identified in key regulators of bile acid flow resulting in a range of cholestatic phenotypes.

GENETIC CAUSES OF CHOLESTASIS

Cholestasis is a feature of a myriad of genetic disorders as summarized in Table 1. Many inborn errors of metabolism, for example, have been identified as causes of cholestasis in infancy, particularly those affecting genes encoding bile

TABLE 1. GENETIC DEFECTS ASSOCIATED WITH CHOLESTASIS

Gene	Disease association	Mechanism of cholestasis
ATP8B1	PFIC	Impaired canalicular membrane transport of bile salts and/or lipids
ABCB11	PFIC	
ABCB4	PFIC	
ABCC2	Dubin-Johnson syndrome	
NR1H4	PFIC	
Myo5B	PFIC, MVID	Failure of intracellular protein trafficking, altered apical/basolateral polarity
VPS33B *VIPAS39*	Arthrogryposis-renal dysfunction-cholestasis syndrome	
SCYL1	SCAR21	
AP1S1	MEDNIK syndrome	
JAG1 *NOTCH 2*	ALGS	Impaired bile duct development
CFTR	Cystic fibrosis	Altered cholangiocyte secretion
TJP2	PFIC	Altered tight junction integrity
CLDN1	NISCH syndrome	
SERPINA1	Alpha-1-antitrypsin deficiency	Misfolded AAT protein in liver
GALT	Galactosemia	Inborn errors of amino acid and lipid metabolism
FAH	Tyrosinemia	
SLC25A13	Citrin deficiency	
LIPA	Wolman disease	
NPC1	Niemann-Pick disease type C1	
GBA	Gaucher disease type 2	
HSD3B7	CBAS1	Inborn errors of bile salt synthesis and conjugation
AKR1D1	CBAS2	
CYP7B1	CBAS3, SPG5A	
AMACR	CBAS4	
ABCD3	CBAS5, Zellweger syndrome	
ACOX2	CBAS6	
CYP27A1	Cerebrotendinous xanthomatosis	
EPHX1	Familial hypercholanemia	
BAAT	Familial hypercholanemia	

acid synthetic enzymes. In addition, several multisystem genetic diseases, such as Alagille syndrome (ALGS) and cystic fibrosis, exhibit cholestasis as one of many diverse manifestations. There is a range of pathophysiological disturbances that lead to cholestasis in these diverse genetic diseases. For example, the cholangiopathy in ALGS results from aberrant bile duct development. The remainder of this review focuses on progressive familial intrahepatic cholestasis (PFIC), benign recurrent intrahepatic cholestasis (BRIC), and intrahepatic cholestasis of pregnancy (ICP), a spectrum of diseases arising from defects in hepatocellular bile formation and flow. Complete or near-complete disruption of the affected genes results in PFIC, a severe form of intrahepatic cholestasis that presents in early childhood and progresses to end-stage liver disease. In contrast, mutations resulting in partial deficiency of select PFIC-associated genes predispose to BRIC or ICP, milder forms of the disease that present later in life.

PFIC

Traditionally, there have been three main subtypes of PFIC resulting from mutations in *ATP8B1* (FIC), *ABCB11* (BSEP), and *ABCB4* (MDR3) (Table 2 and Fig. 2). Because of the expanding use of next-generation sequencing technology, mutations causing three additional subtypes of PFIC have been identified in the last decade. Specifically, mutations in tight junction protein 2 (*TJP2*), FXR (*NR1H4*), and myosin Vb (*MYO5B*) have been identified as novel causes of genetic cholestasis.[3-6] A recent analysis of families with cholestatic diseases resembling PFIC has led to the identification of other novel mutations associated with cholestasis (*KIF12, PPM1F, USP53, LSR*, and *WDR83OS*), thereby expanding our understanding of the mechanistic underpinnings of intrahepatic genetic cholestasis.[7] The PFIC subtypes resulting from defects in FIC, BSEP, and MDR3 have been historically identified numerically as PFIC 1 through 3, respectively. With the rapidly increasing number of genes linked to PFIC, this numerical classification is no longer preferred. Instead, molecular classification, in which the subtype is identified by the genetic defect, is increasingly favored.

The pathophysiology underlying "classic" PFIC (i.e., FIC, BSEP, and MDR3 deficiency) is fundamentally similar

TABLE 2. CLINICAL MANIFESTATIONS OF PFIC

Mutated Gene	Gene Product	Consequence of Defect	Age at Symptom Onset	Rate of Progression	GGT	Extrahepatic Manifestations	Manifestations of Partial Gene Deficiency
ATP8B1	FIC1	Altered canalicular aminophospholipid transport, impaired hepatic bile formation	Infancy	Moderate	Low or normal	Elevated sweat chloride, delayed puberty, watery diarrhea, hearing loss, pancreatitis	BRIC1, ICP
Abcb11	BSEP	Decreased canalicular bile salt secretion, impaired bile formation	Infancy	Moderate	Low or normal	Uncommon	BRIC2, ICP, drug-induced cholestasis
Abcb4	MDR3	Decreased canalicular phospholipid transport, impaired bile formation	Childhood/early adulthood	Variable, moderate	Elevated	Uncommon	Low phospholipid-associated cholelithiasis ICP Drug-induced liver injury
TJP2	TJP2	Deficiency of TJP, leakage of bile through paracellular space into liver parenchyma	Infancy	Rapid	Normal or slightly elevated	Neurological, respiratory	Benign familial hypercholanemia
NR1H4	FXR	Impaired suppression of bile salt synthesis, decreased canalicular secretion	Early infancy	Very rapid	Low or normal	Vitamin K-independent coagulopathy	Gallstone formation ICP
MYO5B	MYO5B	Abnormal intracellular trafficking, decreased localization of BSEP to canalicular membrane	Infancy	Variable, slow	Low or normal	MVID causing severe watery diarrhea	Unknown

in that these subtypes result from defects in the major canalicular membrane transporters (Fig. 2). The discovery of mutations in *TJP2*, *NR1H4*, and *MYO5B* has broadened our understanding of the range of pathophysiological disturbances that lead to intrahepatic cholestasis. *TJP2*, for example, encodes a TJP, the loss of which allows leakage of biliary components from the canaliculi into hepatocytes. Disruption of *NR1H4* (FXR) leads to severe cholestasis through multiple mechanisms, chief of which are the loss of feedback inhibition of bile acid synthesis and impaired induction of canalicular membrane transporters. MYO5B is involved in the trafficking of BSEP to the canalicular membrane, the dysfunction of which impairs canalicular bile acid secretion.

Clinically, PFIC typically presents in infancy with jaundice and pruritus and, if left untreated, progresses to cirrhosis. The pruritus is often intense and intractable. The subtypes of PFIC can be differentiated to some degree clinically based on the age of onset and rapidity of progression, as well as other distinct clinical features (Table 2). Although PFIC is classically associated with low/normal serum gamma glutamyl transferase (GGT), the exception is MDR3 deficiency, which exhibits high GGT. Interestingly, mutations in MYO5B were first identified as the cause of microvillus inclusion disease (MVID), and the association with low-GGT cholestasis is a relatively recent discovery. Certain PFIC subtypes confer an increased risk for hepatocellular carcinoma in childhood, most notably BSEP deficiency. Additional differentiating features of the PFIC subtypes are summarized in Table 2.

BRIC

BRIC represents a less severe form of PFIC typically characterized by recurrent episodes of cholestasis triggered by infections or medications. There are two subtypes of BRIC (BRIC 1 and BRIC2) caused by mutations in *ATP8B1* and *ABCB11*, respectively[8,9] (Table 2). In contrast with PFIC, BRIC typically manifests in late childhood or early adulthood with mild cholestatic symptoms, classically pruritus. Liver histology is typically normal between episodes of cholestasis, and progression to advanced liver disease is rare.

ICP

ICP is characterized by the onset of cholestatic symptoms during pregnancy. Mutations in several genes,

including *ABCB4*, *ABCB11*, *ABCC2*, *ATP8B1*, *TJP2*, and *NR1H4,* have been linked to ICP, with mutations in *ABCB4* being most common[10-12] (Table 2). The classic presentation is pruritus beginning in the third trimester. Typical laboratory findings include elevated aminotransferases and high levels of serum bile acids. The management centers on controlling symptoms, typically using ursodeoxycholic acid for pruritus. The definitive treatment is delivery, with most cases of ICP resolving completely in the postpartum period. Although the condition is thought to have little impact on the long-term health of the mother, ICP is associated with an increased risk for serious pregnancy complications, including fetal distress or intrauterine death.

CONCLUSION

Due to advances in next-generation sequencing, tremendous progress has been made in recent years toward discovering all of the genes responsible for various forms of genetic cholestasis. Still, nearly a third of patients with PFIC-like syndromes do not harbor mutations in any of the known PFIC-associated genes. As such, there is considerably more work to be done to fully characterize this family of disorders at a genetic level.

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REFERENCES

1) Chiang JYL, Ferrell JM. Bile acid biology, pathophysiology, and therapeutics. Clin Liver Dis (Hoboken) 2020;15:91-94.

2) Kliewer SA, Mangelsdorf DJ. Bile acids as hormones: the FXR-FGF15/19 pathway. Dig Dis 2015;33:327-331.

3) Sambrotta M, Strautnieks S, Papouli E, et al. Mutations in TJP2 cause progressive cholestatic liver disease. Nat Genet 2014;46:326-328.

4) Gomez-Ospina N, Potter CJ, Xiao R, et al. Mutations in the nuclear bile acid receptor FXR cause progressive familial intrahepatic cholestasis. Nat Commun 2016;7:10713.

5) Qiu YL, Gong JY, Feng JY, et al. Defects in myosin VB are associated with a spectrum of previously undiagnosed low gamma-glutamyltransferase cholestasis. Hepatology 2017;65:1655-1669.

6) 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.

7) Maddirevula S, Alhebbi H, Alqahtani A, et al. Identification of novel loci for pediatric cholestatic liver disease defined by KIF12, PPM1F, USP53, LSR, and WDR83OS pathogenic variants. Genet Med 2019;21:1164-1172.

8) van Mil SWC, 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.

9) Klomp LW, Vargas JC, van Mil SW, et al. Characterization of mutations in ATP8B1 associated with hereditary cholestasis. Hepatology 2004;40:27-38.

10) Wasmuth HE, Glantz A, Keppeler H, et al. Intrahepatic cholestasis of pregnancy: the severe form is associated with common variants of the hepatobiliary phospholipid transporter ABCB4 gene. Gut 2007;56:265-270.

11) van Mil SWC, Milona A, Dixon PH, et al. Functional variants of the central bile acid sensor FXR identified in intrahepatic cholestasis of pregnancy. Gastroenterology 2007;133:507-516.

12) Dixon PH, Sambrotta M, Chambers J, et al. An expanded role for heterozygous mutations of ABCB4, ABCB11, ATP8B1, ABCC2 and TJP2 in intrahepatic cholestasis of pregnancy. Sci Rep 2017;7:11823.