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Progressive familial intrahepatic cholestasis: From childhood to adulthood

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Abstract

Progressive familial intrahepatic cholestasis (PFIC) is a heterogeneous group of inherited cholestatic liver disorders characterized by impaired bile formation and secretion. Although traditionally classified into numbered subtypes, PFIC is now increasingly understood within a mechanism-based framework involving defects in canalicular transport, membrane lipid homeostasis, tight junction integrity, intracellular trafficking, and bile acid regulatory pathways. This evolving classification better accounts for the marked phenotypic variability, overlapping clinical presentations, and expanding genetic spectrum of the disease. From a clinical perspective, PFIC typically presents with cholestasis, pruritus, and growth impairment in infancy or early childhood. However, accumulating evidence indicates that disease expression may extend into adolescence and adulthood, with milder or atypical phenotypes increasingly recognized. Biochemical stratification, particularly based on gamma-glutamyl transferase levels, remains a key diagnostic entry point, but genetic testing is required for definitive diagnosis. Genotype-phenotype correlations, especially in *ABCB11*- and *ABCB4*-related diseases, have important implications for prognosis and malignancy risk. Furthermore, recent advances in pathophysiology have informed the development of targeted therapies, including ileal bile acid transporter inhibitors, while surgical approaches and liver transplantation remain essential in advanced disease. This review provides a comprehensive overview of the molecular mechanisms, clinical features, diagnostic strategies, and current as well as emerging therapeutic approaches for PFIC.

Key Words: Bile acid transport; Cholestasis; Genotype-phenotype correlation; Ileal bile acid transporter inhibitors; Genetic diagnosis

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Core Tip: Progressive familial intrahepatic cholestasis (PFIC) is a genetically and mechanistically diverse group of cholestatic liver disorders whose clinical spectrum spans from childhood well into adulthood. The disease classification has evolved from a numerical system to a mechanism-based framework encompassing defects in canalicular transport, membrane stability, tight junction integrity, intracellular trafficking, and bile acid regulation. This comprehensive review underscores the significance of integrating early genetic testing with biochemical patterns, particularly gamma-glutamyl transferase levels, for accurate diagnosis. Genotype-phenotype correlations and extrahepatic manifestations are essential for prognostic assessment and individualized management. Emerging targeted therapies, including ileal bile acid transporter inhibitors, support a mechanism-driven approach to PFIC.

INTRODUCTION

Progressive familial intrahepatic cholestasis (PFIC) comprises a genetically and mechanistically heterogeneous group of autosomal recessive cholestatic liver disorders caused by defects affecting key components of hepatobiliary bile formation and secretion[1,2]. The condition was first described by Clayton *et al*[3] in 1965 as "Byler disease". The estimated incidence ranges from 1 in 50000 to 1 in 100000 live births, with no significant sex predilection[2,4].

Although PFIC was historically considered a disease exclusive to infancy and early childhood, the increasing utilization of genetic testing has revealed that it can also manifest in adolescence and adulthood with a broader clinical spectrum[4,5]. Concurrently, advances in molecular genetics have expanded the disease classification from the original three types to at least thirteen genetically defined subtypes, reflecting the heterogeneity of the underlying pathogenic mechanisms as reflected in multiple gene-specific entries in the Online Mendelian Inheritance in Man (OMIM) database[1,6].

Clinically, PFIC is no longer regarded as a uniform disorder limited to jaundice, pruritus, and growth retardation. Rather, it encompasses a wide spectrum of cholestatic diseases with variable severity, ranging from intermittent or mild cholestasis to rapidly progressive liver failure[4,7]. Crucially, PFIC is associated with

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severe long-term complications, including cirrhosis, hepatocellular carcinoma (HCC), and, in some cases, cholangiocarcinoma, affecting both pediatric and adult populations[1,4].

Emerging evidence indicates that variants in PFIC-related genes may also contribute to a broader range of cholestatic phenotypes beyond classical PFIC. These include adult-onset low-phospholipid-associated cholelithiasis (LPAC), benign recurrent intrahepatic cholestasis (BRIC), drug-induced cholestasis, intrahepatic cholestasis of pregnancy (ICP), and cryptogenic cholestatic liver disease[4,5]. In these conditions, PFIC-related variants may act as disease modifiers or susceptibility factors, contributing to phenotypic variability and disease severity[2,6].

From a therapeutic perspective, management of PFIC remains challenging. Conventional treatments, including ursodeoxycholic acid (UDCA) and antipruritic medications, often provide limited efficacy, particularly in severe disease[7,8]. In recent years, novel pharmacologic agents targeting enterohepatic bile acid circulation, particularly selective ileal bile acid transporter (IBAT) inhibitors, have demonstrated promising clinical and biochemical improvements, marking a significant advance in the treatment landscape[9,10].

Despite these developments, the natural history of PFIC remains unfavorable in many. Fewer than half of individuals with PFIC1 retain their native liver into adulthood, and approximately 70% of patients ultimately require surgical biliary diversion or liver transplantation[11,12].

Pruritus represents one of the most debilitating clinical features of PFIC, significantly impairing quality of life[13]. In pediatric patients, severe and persistent itching often occurring both day and night can be profoundly distressing and may constitute an indication for liver transplantation even in the absence of advanced hepatic dysfunction[7].

In light of this expanding clinical and genetic spectrum, it has become increasingly clear that PFIC can no longer be adequately understood through a purely phenotype-based or numerical classification. Instead, current evidence supports a mechanism-based framework in which disease manifestations arise from defects affecting distinct yet interconnected components of hepatobiliary

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physiology[1,4]. These include canalicular transport systems, membrane lipid composition, tight junction integrity, intracellular trafficking pathways, and bile acid-regulated transcriptional networks.

This mechanistic perspective not only provides a more coherent explanation for the marked phenotypic heterogeneity observed across PFIC subtypes, but also establishes the foundation for genotype-driven diagnosis and targeted therapeutic strategies[5,7]. Accordingly, a detailed understanding of the molecular architecture underlying PFIC is essential for accurate disease classification, prognostic assessment, and the development of personalized treatment approaches.

Search strategy and study selection

Literature for this narrative review was retrieved through a structured, comprehensive search of the PubMed/MEDLINE, Scopus, and Web of Science. Articles published in English up to March 2026 were screened using combinations of the following terms: "Progressive familial intrahepatic cholestasis", "PFIC", "*ABCB11*", "*ABCB4*", "*ATP8B1*", "*TJP2*", "*NR1H4*", "*MYO5B*", "*USP53*", "*KIF12*", "*ZFYVE19*", "*VPS33B*", "*PSKH1*", "cholestasis", "bile acid transport" and "IBAT inhibitors". The reference lists of all identified relevant articles were manually reviewed to identify additional studies. Priority was given to recent reviews, clinical guidelines, cohort studies, clinical trials, and key mechanistic papers. Given the narrative design of this review, formal evidence grading was not performed; however, structural emphasis was rigorously placed on clinically relevant and high-quality peer-reviewed publications.

MOLECULAR ARCHITECTURE OF PFIC: A MECHANISM-BASED MODULAR FRAMEWORK

PFIC comprises a heterogeneous group of monogenic disorders characterized by defective hepatocellular bile formation and secretion[14]. Although traditionally classified numerically (PFIC1-PFIC13; OMIM phenotypic series), contemporary hepatology supports a mechanism-based framework that reflects disruption of distinct yet interconnected components of the hepatobiliary transport machinery[15].

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This modular perspective offers a more coherent explanation for the marked phenotypic variability, genotype-phenotype overlap, and the expanding spectrum of PFIC-related disorders[2,14]. The specific genes implicated in PFIC pathogenesis and their corresponding molecular mechanisms are structurally summarized in Table 1 and illustrated in Figure 1.

Canalicular transport defects

The adenosine triphosphate (ATP)-binding cassette transporter family constitutes a large superfamily of transmembrane proteins with numerous members that form the core components of the hepatobiliary transport system and play a critical role in the canalicular secretion of biliary constituents. Among these, the transporters most closely related to hepatobiliary physiology include *ABCB11* [bile salt export pump (BSEP)], *ABCB4* [multidrug resistance protein-3 (MDR3)], *ABCC2* (multi-drug resistance protein 2), and the *ABCG5/ABCG8* heterodimer. Within the PFIC spectrum, the primary genetic determinants of canalicular transport defects are *ABCB11* and *ABCB4*[16,17].

ABCB11 encodes the BSEP, a canalicular membrane transporter in hepatocytes that represents the rate-limiting step in the secretion of bile acids from the hepatocyte cytoplasm into the canalicular lumen[18]. Biallelic (homozygous or compound heterozygous) loss-of-function variants lead to intracellular bile acid accumulation, resulting in marked hepatocellular toxicity, severe early-onset cholestasis, and, in advanced cases, an increased risk of HCC in childhood[17,19,20]. *ABCB11*-associated phenotypes are not restricted to PFIC2; rather, they form a spectrum largely determined by residual BSEP activity and may include milder or episodic cholestatic conditions such as BRIC type 2 as well as susceptibility states such as ICP (Table 1). Accordingly, contemporary concepts support a mechanism-based framework for PFIC rather than a purely numerical classification[14,17,19,21,22].

From a genotype-phenotype perspective, protein-truncating variants such as nonsense/frameshift- are consistently associated with the most severe and early-onset presentations of the disease. Conversely, numerous missense variants retain

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partial residual activity through defects in protein folding, stability, or canalicular trafficking and therefore produce a more variable or comparatively milder clinical spectrum[17,19,21,23-27]. Although specific recurrent missense alleles [*e.g.*, p.(Glu297Gly) and p.(Asp482Gly)] are frequently categorized as milder genotypes, large cohort data indicate that allelic combination is a major determinant of disease severity, and the presence of a "mild" missense variant in trans with a protein-truncating variant may still result in a severe phenotype[17,27,28]. Increasing evidence further suggests that heterozygous *ABCB11* variants, while not typically causing a fully penetrant Mendelian disorder on their own, may act as susceptibility factors for cholestasis under environmental stressors such as hormones or drugs. This supports a gene-environment interaction model in which reduced transporter reserve predisposes to cholestasis when physiological demand increases[17,22,28].

ABCB4 encodes MDR3, an ATP-dependent phospholipid floppase localized to the hepatocyte canalicular membrane that translocates phosphatidylcholine from the inner to the outer leaflet of the lipid bilayer into bile[14]. These phospholipids form mixed micelles with bile acids, thereby buffering the detergent activity of hydrophobic bile salts and preserving biliary epithelial integrity[17,29].

Biallelic (homozygous or compound heterozygous) loss-of-function variants in *ABCB4* lead to reduced phosphatidylcholine secretion, culminating in the formation of low-phospholipid bile. This impairment in mixed micelle formation increases exposure of the biliary epithelium to unbuffered, detergent bile acids, resulting in cholangiocellular vulnerability[30-32]. Importantly, this pathophysiology reflects a primary defect in bile composition and phospholipid homeostasis, rather than a direct impairment of bile acid export. This mechanistic distinction clearly differentiates *ABCB4* deficiency from other forms of canalicular cholestasis driven by primary bile acid transport failure[14,17,32].

ABCB4 variants are not confined to PFIC3 but instead define a broad biological continuum. Depending on the level of residual MDR3 activity, the phenotypic spectrum ranges from severe fibrocholestatic liver disease to LPAC, ICP, and adult-onset cholangiopathic phenotypes. Current evidence indicates that phenotypic severity is largely determined by zygosity and variant type[14,17,30,31].

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From a genotype-phenotype perspective, protein-truncating variants are generally associated with more profound phosphatidylcholine deficiency. In contrast, many missense variants produce partial defects in MDR3 folding or canalicular targeting, allowing variable residual phospholipid secretion. This variability in micellar stability and bile detoxification capacity likely underlies the marked phenotypic heterogeneity observed in *ABCB4*-related disease[14,17,30,33,34].

Heterozygous *ABCB4* variants are often clinically silent in isolation; however, phenotypic expression may emerge under conditions that further limit micellar buffering capacity, such as pregnancy, estrogen exposure, infections, or cholestatic medications. These observations support a gene-environment interaction model in which *ABCB4*-related disorders reflect an underlying fragility of bile composition rather than a fixed binary defect[17,21,31] (Figure 1).

This schematic illustrates the principal molecular pathways involved in hepatocellular bile formation and secretion and summarizes the major mechanistic categories underlying PFIC. Canalicular transport defects (*e.g.*, *ABCB11*, *ABCB4*, *ABCC2*, *ABCG5/ABCG8*) impair bile acid, phospholipid, and organic anion secretion, whereas defects in membrane lipid homeostasis (*ATP8B1*) compromise canalicular membrane stability. Tight junction abnormalities (*TJP2*, *USP53*) disrupt epithelial barrier integrity and promote paracellular bile leakage, while intracellular trafficking and polarity defects (*MYO5B*, *VPS33B*, *ZFYVE19*) impair canalicular transporter localization. Nuclear receptor dysfunction (*NR1H4*) alters bile acid synthesis and transporter regulation, whereas defects in enterohepatic circulation (*SLC51A/SLC51B*) disrupt bile acid recycling.

Membrane lipid homeostasis and canalicular stability

ATP8B1 encodes familial intrahepatic cholestasis protein 1 (FIC1), a member of the P4-type adenosine triphosphatase family that functions as a phospholipid flippase responsible for maintaining canalicular membrane lipid asymmetry and stability[35-37]. Biallelic pathogenic variants in *ATP8B1* cause a phenotypic spectrum ranging from BRIC type 1 (BRIC1) to PFIC1. Unlike BSEP deficiency, *ATP8B1* disease is

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primarily considered a disorder of membrane integrity rather than a direct bile salt export defect[38,39].

At the molecular level, *ATP8B1* deficiency renders the canalicular membrane more susceptible to bile salt-induced injury, leading to hepatocellular damage and secondary disturbances in farnesoid X receptor (FXR)-regulated bile acid homeostasis[39]. Because *ATP8B1* is expressed in multiple extrahepatic tissues, affected patients may present with chronic diarrhea, pancreatitis, growth failure, and sensorineural hearing loss; experimental animal models have further suggested possible systemic and neurodevelopmental involvement[35,40,41]. These features provide important clinical clues for distinguishing PFIC1 from other causes of low-gamma-glutamyl transferase (GGT) cholestasis.

The mutational spectrum of *ATP8B1* is highly heterogeneous and includes missense, nonsense, frameshift, splice-site, and structural variants such as multi-exonic deletions and duplications. Residual FIC1 function is a major determinant of clinical severity: Missense variants tend to be associated with the milder BRIC1 phenotype, whereas truncating variants are more commonly linked to PFIC1[42-45]. Nevertheless, marked intrafamilial variability and phenotypic overlap indicate additional contributions from modifier genes, epigenetic influences, and environmental factors[41]. Notably, selected synonymous variants may also exert pathogenic effects through aberrant splicing or transcript instability when functionally validated[45].

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Tight junction and epithelial barrier dysfunction

The integrity of hepatocellular tight junctions is critical for maintaining the blood-bile barrier and preserving hepatocyte polarity. Within the PFIC spectrum, tight junction-related cholestasis is primarily represented by *TJP2* (PFIC4) and *USP53* (PFIC7), whereas *CLDN1* deficiency constitutes a PFIC-mimicking syndromic cholangiopathy rather than a classical PFIC subtype[14,46-48].

TJP2, also known as zona occludens-2, is an important cytoplasmic scaffold protein of the tight junction complex in epithelial cells. It connects transmembrane tight junction proteins to the actin cytoskeleton, thereby maintaining intercellular

barrier integrity. In the liver, tight junctions between hepatocytes form the bile-blood barrier, thereby preventing leakage of biliary components into the hepatic parenchyma. The integrity of this structure is essential for maintaining hepatocyte polarity and the physiological regulation of bile secretion[49-51].

Biallelic loss-of-function mutations in *TJP2* destabilize the tight junction complex between hepatocytes, allowing bile acids and other toxic biliary components to leak into the intercellular space through the paracellular pathway, ultimately leading to hepatocellular injury and progressive cholestasis. In addition, *TJP2* deficiency disrupts the proper canalicular localization of claudin-1, further compromising the integrity of the hepatobiliary barrier[49,50].

Experimental models indicate that the consequences of *TJP2* loss extend beyond barrier dysfunction; *TJP2*-deficient livers demonstrate dilation of bile canaliculi, reduced microvillus density, and altered radixin distribution, along with decreased expression of key bile acid transporters such as *ABCB11*/BSEP. Together, these alterations impair bile acid excretion, promote intracellular bile acid accumulation, and contribute to progressive cholestatic liver injury[51].

The phenotypic spectrum of *TJP2*-related disease extends beyond classical biallelic loss-of-function mutations. Heterozygous *TJP2* variants have been described in individuals with milder cholestatic phenotypes and may act as genetic susceptibility factors for ICP[49,52]. A representative example is the p.(Val48Ala) founder mutation identified in the Old Order Amish population. This missense variant, located in the PDZ1 (postsynaptic density protein-95/discs large/zona occludens-1) domain of *TJP2*, impairs claudin binding while retaining partial protein function and is associated with familial hypercholanemia-1. The phenotype is characterized by incomplete penetrance and variable clinical severity, and in some cases liver disease may improve with age. In certain individuals, the phenotype may be further modified by digenic inheritance involving *BAAT*[53]. In addition, because *TJP2* is widely expressed in epithelial tissues, rare extrahepatic manifestations such as hearing loss have also been reported[54].

Recent studies indicate that *TJP2* functions not only as a structural component of tight junctions but also as a regulator of Hippo signaling. Loss of *TJP2* promotes

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nuclear translocation of the Hippo pathway effectors yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) and activation of proliferative transcriptional programs; in experimental models, *TJP2* deficiency has been shown to induce hepatocyte-to-cholangiocyte transdifferentiation through Hippo pathway dysregulation[55]. Aberrant activation of YAP/TAZ signaling is a recognized driver of HCC development, suggesting a potential mechanistic link between *TJP2* deficiency and tumorigenesis[56]. Notably, HCC reported in *TJP2*-related disease has predominantly occurred in patients with biallelic truncating variants causing complete loss of *TJP2* function, suggesting that severe disruption of tight junction integrity and chronic bile acid-mediated hepatocyte injury may contribute to hepatocarcinogenesis[46,57-59].

USP53, a protein expressed in epithelial tissues that contributes to the stabilization of the tight junction complex and the maintenance of hepatocyte polarity[60,61]. Although *USP53* belongs to the deubiquitinating enzyme family, it lacks the catalytic histidine residue required for classical enzymatic activity, suggesting that its function is predominantly structural and regulatory rather than enzymatic[62]. Experimental studies have demonstrated that *USP53* interacts with tight junction components, particularly *TJP2*, thereby contributing to the maintenance of epithelial barrier integrity; loss of this protein disrupts junctional organization and may lead to intrahepatic cholestasis characterized by low or normal GGT levels[47,61,62].

More recent experimental data indicate that the consequences of *USP53* deficiency extend beyond tight junction instability. In particular, loss of *USP53* has been shown to impair the canalicular trafficking of the BSEP (*ABCB11*) through disruption of the *MYO5B*- and *Rab11*-mediated recycling endosome pathway, resulting in intracellular retention of BSEP and reduced bile acid secretion. These findings suggest that the pathogenesis of *USP53*-related cholestasis cannot be explained solely by tight junction destabilization but likely reflects a more complex mechanism involving defects in the intracellular trafficking of apical membrane transporters[63]. Notably, current clinical data indicate that genotype-phenotype

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correlations remain poorly defined, suggesting that additional genetic or environmental modifiers may influence disease severity[60,64-66].

Nuclear receptor and transcriptional regulation

Nuclear receptors involved in bile acid signaling play a critical role in maintaining hepatobiliary homeostasis. Among these regulators, *NR1H4* is the principal gene directly associated with PFIC in this functional group. *NR1H4* encodes the FXR, a bile acid–activated nuclear receptor that functions as a key transcription factor regulating bile acid synthesis, hepatocellular transport, and enterohepatic circulation[67-69].

In hepatocytes, activation of FXR regulates the expression of several target genes involved in bile acid homeostasis. FXR induces the expression of the canalicular bile acid transporter (BSEP/*ABCB11*), thereby promoting the secretion of bile acids from hepatocytes into the bile canaliculi. In addition, FXR suppresses bile acid synthesis by inducing small heterodimer partner, which inhibits transcription of *CYP7A1*, the rate-limiting enzyme of bile acid synthesis. This coordinated negative feedback mechanism prevents intracellular bile acid accumulation and protects hepatocytes from bile acid-mediated toxicity[67,70].

Biallelic pathogenic variants in *NR1H4* disrupt FXR-mediated transcriptional regulation, leading to impaired control of bile acid synthesis and secretion. In the absence of functional FXR signaling, bile acid synthesis cannot be adequately suppressed while canalicular export is simultaneously reduced, resulting in rapid intracellular accumulation of toxic bile acids and early-onset cholestatic liver injury. To date, approximately 15 patients with *NR1H4*-related disease have been reported worldwide, harboring 16 different pathogenic variants. However, the limited number of reported cases currently precludes robust genotype-phenotype correlations[71,72].

Intracellular trafficking, epithelial polarity and cytoskeletal regulation

VPS33B is a member of the Sec1/Munc18 protein family and functions as a trafficking protein involved in intracellular vesicular transport, membrane fusion,

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protein sorting, and the maintenance of apical-basolateral polarity in polarized cells. *VPS33B* interacts with soluble N-ethylmaleimide-sensitive-factor attachment protein receptor proteins and forms a complex with *VIPAS39*/*VIPAR*, enabling proper targeting of cargo proteins in polarized epithelial cells, hepatocytes, and megakaryocytes[73-75]. Consequently, *VPS33B* dysfunction results in a multisystem phenotype affecting the liver, renal tubules, epidermis, and platelets[74-78]. In the liver, *VPS33B* plays a crucial role in maintaining hepatocyte polarity. Experimental studies have shown that loss of *VPS33B* leads to mislocalization of apical membrane proteins and canalicular transport systems, while basolateral protein localization remains relatively preserved[76,79,80]. Current evidence suggests that *VPS33B*-related disorders represent a broad phenotypic spectrum ranging from severe arthrogryposis-renal dysfunction-cholestasis (ARC) syndrome to milder or incomplete ARC forms and autosomal recessive keratoderma-ichthyosis-deafness (ARKID) syndrome. Clinical variability appears to be largely determined by mutation type and residual protein function.[75,77,81-83]. Notably, the presence of the p.(Gly131Glu) missense variant in at least one allele has been proposed to be associated with the milder cutaneous-dominant phenotype observed in ARKID syndrome[77,81,83].

Beyond *VPS33B*, accumulating evidence indicates that defects in intracellular trafficking pathways regulating epithelial polarity and cytoskeletal organization also contribute to cholestatic liver disease. In this context, *ZFYVE19* encodes zinc finger *FYVE*-type containing protein 19, also known as abscission/NoCut checkpoint regulator, which participates in the regulation of the final stage of cytokinesis. *ZFYVE19* interacts with vacuolar protein sorting-4 and functions as a regulatory component of the abscission checkpoint, ensuring accurate chromosomal segregation during cell division[84-86]. In addition, *ZFYVE19* has been implicated in primary cilium formation and cellular polarity-associated pathways[85,86].

Genetic studies have demonstrated that biallelic loss-of-function variants in *ZFYVE19* are associated with hereditary cholestatic liver disease characterized by progressive hepatobiliary dysfunction[86-88]. Current data support the concept that the underlying pathogenic mechanism is loss of *ZFYVE19* protein function[86,89,90].

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Genotype-phenotype correlations remain poorly defined, as patients carrying the same pathogenic variant may exhibit variable clinical outcomes, suggesting the presence of additional genetic or environmental modifiers[88]. Experimental studies further support this mechanism; in *ZFYVE19* -/- mouse models, genetic therapy restoring *ZFYVE19* expression, together with bile acid-modulating treatments, has been shown to ameliorate hepatobiliary injury and attenuate liver fibrosis[89,90].

MYO5B encodes myosin Vb, an actin-based motor protein that regulates intracellular vesicular trafficking and recycling endosome transport in polarized epithelial cells. *MYO5B* interacts with Rab family GTPases, particularly *Rab11* and *Rab8*, and plays a central role in the targeting and recycling of apical membrane proteins[91,92]. In hepatocytes, *MYO5B* regulates the vesicular trafficking and canalicular localization of key bile transporters, including BSEP and MDR3, through *Rab11*-dependent recycling pathways[93,94]. Disruption of *MYO5B* function leads to mislocalization of these canalicular transport proteins and impaired bile secretion, ultimately resulting in cholestasis[93,95,96].

Accumulating evidence points to *MYO5B*-related disorders comprise a broad phenotypic spectrum. Clinical manifestations range from isolated intestinal disease, such as microvillus inclusion disease, to predominant cholestatic liver disease or mixed intestinal-hepatic phenotypes. Genotype-phenotype correlations appear to depend largely on the type of variant, its location within the protein, and the degree of residual *MYO5B* function[93-98]. Variants causing complete loss of protein function are more commonly associated with severe intestinal disease, whereas variants allowing partial protein activity may preferentially impair hepatocellular transporter trafficking and present with isolated cholestatic liver disease[95]. Recent experimental studies further suggest that certain *MYO5B* variants, including heterozygous variants, may exert dominant-negative or toxic gain-of-function effects on hepatocellular trafficking pathways, thereby impairing canalicular transporter localization and contributing to intrahepatic cholestasis[93,96].

Enterohepatic circulation components

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In addition to defects affecting intracellular trafficking and hepatocellular polarity, disturbances in bile acid transport systems may also contribute to cholestatic phenotypes. The organic solute transporter α/β (OSTα/β), encoded by *SLC51A* and *SLC51B*, is a heterodimeric bile acid transporter expressed in the liver, intestine, and kidney. Localized to the basolateral membrane of hepatocytes and enterocytes, OSTα/β mediates the efflux of bile acids into the portal circulation and plays an important role in maintaining bile acid homeostasis[99-101]. To date, only a few cases associated with *SLC51A* have been reported. The first confirmed case involved a homozygous p.(Gln186Ter) variant presenting with early-onset diarrhea and cholestasis[102]. Three additional related patients carrying a homozygous p.(Ile282Thr) variant were reported with early-onset fat malabsorption and fat-soluble vitamin deficiency[103]. Although this variant was classified as a variant of uncertain significance, the cases were considered part of the broader clinical spectrum associated with *SLC51A*[103]. In addition, two siblings with biallelic *SLC51B* variants have been reported, presenting with chronic diarrhea, fat-soluble vitamin deficiency, and biochemical features of cholestatic liver disease, further supporting the role of the OSTα/β complex in bile acid homeostasis[104].

Recently identified PFIC genes

KIF12 encodes kinesin family member 12, a microtubule-dependent motor protein involved in intracellular cargo transport. Kinesin proteins regulate vesicular and protein trafficking along microtubules and play essential roles in intracellular transport, cytoskeletal organization, and the maintenance of epithelial cell polarity[105,106]. A significant proportion of the reported pathogenic variants have been shown to localize within the kinesin motor domain[105,107]. The molecular mechanism underlying *KIF12*-associated cholestasis has not yet been fully elucidated. However, current evidence suggests that *KIF12* may participate in microtubule-dependent intracellular transport processes that are important for maintaining hepatocyte polarity and the correct localization of canalicular membrane proteins. Consequently, disruption of *KIF12* function may impair

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intracellular trafficking of bile transporter proteins, leading to defective bile secretion and the development of cholestasis[105-109].

SEMA7A encodes semaphorin-7A, a glycosylphosphatidylinositol-anchored membrane protein belonging to the semaphorin family that regulates cell adhesion, immune signaling, and cytoskeletal organization. Through interaction with β1-integrin receptors, *SEMA7A* participates in signaling pathways involved in inflammatory responses and tissue remodeling[110-113]. Experimental studies have demonstrated increased *SEMA7A* expression in fibrotic liver tissue and activation of hepatic stellate cells through integrin-mediated signaling pathways, suggesting a role in liver injury and fibrogenesis[111-114]. Recently, rare variants in *SEMA7A* have been reported in association with severe pediatric cholestatic liver disease presenting with a progressive intrahepatic cholestasis-like phenotype[115]. Although the precise molecular mechanism remains incompletely understood, dysregulation of *SEMA7A*-mediated signaling may contribute to hepatic inflammation, fibrogenesis, and disruption of hepatobiliary homeostasis, thereby promoting cholestasis[110,113-116]. However, due to the very limited number of reported cases, the role of *SEMA7A* in cholestatic liver disease remains to be further clarified.

PSKH1 encodes protein serine kinase H1, a serine/threonine kinase involved in intracellular trafficking and epithelial cell polarity. The protein localizes mainly to the Golgi apparatus and centrosomal compartments and is thought to regulate vesicular transport and membrane protein targeting[117,118]. Experimental evidence also suggests a role in ciliary biology, as homozygous *PSKH1* p.(Leu219Pro) mutant mice develop situs inversus totalis, supporting a defect in ciliary function[119]. Recently, biallelic *PSKH1* variants have been reported in patients with pediatric cholestatic liver disease and hepatorenal involvement, suggesting that *PSKH1*-associated disease represents an autosomal recessive ciliopathy affecting hepatobiliary function[120]. However, due to the limited number of reported cases, genotype-phenotype correlations remain incompletely defined.

Collectively, these findings indicate that disturbances in intracellular trafficking, epithelial polarity, ciliary function, and inflammatory signaling pathways may represent additional mechanisms underlying PFIC-like cholestatic liver disease.

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SUBTYPE-SPECIFIC CLINICAL PROFILES

PFIC comprises multiple genetically defined subtypes, each associated with distinct molecular mechanisms, clinical features, and disease trajectories. Although there is substantial phenotypic overlap across subtypes, recognition of subtype-specific patterns is essential for accurate diagnosis, prognostic stratification, and therapeutic decision-making[4,7].

PFIC1 (ATP8B1 deficiency)

PFIC1 is caused by biallelic pathogenic variants in *ATP8B1*, encoding the phospholipid flippase FIC1, which is critical for maintaining canalicular membrane lipid asymmetry and stability. PFIC1 typically presents itself during infancy with cholestasis, severe pruritus, growth failure, and fat-soluble vitamin deficiency in the setting of low or normal GGT levels[4]. Histopathology often demonstrates bland cholestasis with progressive portal and periportal fibrosis over time[7].

A hallmark of PFIC1 is its prominent extrahepatic involvement, reflecting the broad epithelial expression of *ATP8B1*. Patients frequently develop chronic diarrhea, pancreatitis or pancreatic insufficiency, sensorineural hearing loss, and failure to thrive[121]. These features distinguish PFIC1 from other low-GGT cholestatic disorders and support its classification as a multisystem epithelial disease rather than a purely hepatic condition. Importantly, extrahepatic manifestations may persist or even worsen after liver transplantation, with post-transplant diarrhea and graft steatosis reported in a subset of patients[121].

The clinical course is variable. Although historically considered relatively indolent, recent cohort data indicate that fibrosis may develop early in some individuals. Despite phenotypic variability, long-term native liver survival remains limited, with only approximately 44% of patients retaining their native liver into late adolescence or adulthood[12].

PFIC2 (ABCB11 deficiency)

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PFIC2 results from biallelic pathogenic variants in *ABCB11*, encoding the BSEP, and represents a primary defect in hepatocellular bile acid secretion[4]. It typically presents in the neonatal or early infantile period with severe low-GGT cholestasis, rapidly progressive fibrosis, and early liver failure. Histologically, giant cell hepatitis and marked cholestasis are common, and immunohistochemistry often demonstrates reduced or absent BSEP expression[4].

PFIC2 is characterized by minimal extrahepatic involvement due to the liver-specific expression of *ABCB11*. However, it is one of the most clinically severe PFIC subtypes and shows a strong genotype-phenotype correlation. Residual BSEP function is a major determinant of disease severity. Patients harboring protein-truncating variants or severe loss-of-function genotypes generally demonstrate markedly reduced native liver survival[4,7].

A particularly important feature of PFIC2 is the increased risk of HCC at an early age. Cohort studies have shown that malignancy risk correlates with genotype severity, with substantially higher rates observed in patients with minimal residual BSEP activity[4]. These findings underscore the need for early and structured oncologic surveillance in this subgroup.

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PFIC3 (ABCB4 deficiency)

PFIC3 is caused by biallelic variants in *ABCB4*, encoding MDR3, a phospholipid transporter responsible for phosphatidylcholine secretion into bile. Unlike PFIC1 and PFIC2, the primary defect in PFIC3 is impaired bile composition rather than defective bile acid export, resulting in increased biliary toxicity and cholangiocyte injury[4].

Clinically, PFIC3 is characterized by high-GGT cholestasis and typically presents later in childhood or adolescence, although earlier onset may occur in severe cases. Patients may develop hepatomegaly, pruritus, splenomegaly, portal hypertension, and progressive biliary fibrosis. Histologic findings often reflect chronic cholangiopathy[122].

The phenotypic spectrum of *ABCB4*-related disease extends beyond classical PFIC3. Biallelic variants are usually associated with severe pediatric disease,

whereas heterozygous or milder variants may predispose to adult conditions such as ICP, LPAC, drug-induced cholestasis, and cryptogenic fibrosing cholangiopathy[4,122]. Genotype-phenotype correlations are complex and appear to be influenced by zygosity, variant type, and residual transporter function.

PFIC4 (TJP2 deficiency)

PFIC4 is caused by biallelic pathogenic variants in *TJP2*, a key component of epithelial tight junction complexes[1,46]. The disorder is characterized by low or normal GGT cholestasis resulting from disruption of the hepatocyte bile-blood barrier and leakage of bile acids into the hepatic parenchyma[46,123].

Clinical presentation ranges from neonatal cholestasis to rapidly progressive liver failure requiring transplantation[1,124]. Histologically, PFIC4 shows cholestasis and progressive fibrosis, often culminating in cirrhosis[123]. Though extrahepatic manifestations are relatively uncommon, they may involve neurological or respiratory systems in some patients[1,124].

Recent studies have raised the possibility of an association between *TJP2* deficiency and HCC, possibly mediated through chronic bile acid-induced injury and dysregulation of signaling pathways such as Hippo/YAP[123]. These findings highlight the need for careful long-term surveillance[59,124].

PFIC5 (NR1H4 deficiency)

PFIC5 is an aggressive cholestatic disorder caused by biallelic variants in *NR1H4*, encoding the bile acid-activated nuclear receptor FXR. Loss of FXR function leads to profound dysregulation of bile acid synthesis and transport[75,125].

Clinically, PFIC5 presents in the neonatal period with severe low-GGT cholestasis, markedly elevated alpha-fetoprotein levels, vitamin K-independent coagulopathy, and rapid progression to liver failure[75,125,126]. Additional features may include hypoglycemia, failure to thrive, and hepatosplenomegaly[75]. Despite high intestinal FXR expression, severe diarrhea is not consistently observed[75].

The natural history is characterized by rapid deterioration, with many patients requiring liver transplantation within the first year of life[75,76]. Interpretation of

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persistently elevated alpha-fetal protein levels may be challenging, as they generally reflect hepatocellular injury rather than malignancy in most cases[126,127].

PFIC6 (SLC51A deficiency)

PFIC6 is associated with biallelic variants in *SLC51A*, encoding the OSTα subunit of the OSTα/β bile acid transporter. This transporter mediates basolateral bile acid efflux from enterocytes and hepatocytes, and its deficiency disrupts enterohepatic circulation[2,6].

Clinically, PFIC6 presents with a combined hepatointestinal phenotype, including cholestasis, chronic diarrhea, steatorrhea, growth impairment, and fat-soluble vitamin deficiency[2,6]. This distinguishes it from classical PFIC subtypes driven primarily by hepatocellular defects. Due to the limited number of reported cases, the full clinical spectrum and additional cases and longitudinal studies are needed to better define the clinical spectrum and long-term outcomes[2].

PFIC7 (USP53 deficiency)

PFIC7 is caused by pathogenic variants in *USP53*, a protein involved in tight junction stability and epithelial polarity[128,129]. The disease is relatively rare and may present later in childhood or adolescence compared with classical PFIC subtypes[128,130].

Clinical manifestations include cholestasis with pruritus, and the disease course may be milder in many patients[128]. Approximately 30-35 cases have been reported, with a notable clustering in Middle Eastern populations[128]. Emerging evidence suggests that some patients may respond favorably to IBAT inhibitors, indicating potential therapeutic relevance[131].

PFIC8 (KIF12 deficiency)

PFIC8 is a rare disorder associated with *KIF12*, a kinesin motor protein involved in intracellular trafficking and epithelial polarity[129,132]. It is generally associated with high-GGT cholestasis and presents in early life, although the phenotypic spectrum remains incompletely defined[6,132].

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Notably, atypical presentations have been described, including cases without overt cholestasis but with chronic liver disease and pancreatitis, suggesting that *KIF12*-related disease may extend beyond classical cholestatic phenotypes[129]. More aggressive disease courses progressing to end-stage liver disease and early liver transplantation have also been reported[133].

PFIC9 (ZFYVE19 deficiency)

PFIC9 is caused by biallelic variants in *ZFYVE19* and is increasingly recognized as a cholangiociliopathy[6,85]. It is characterized by high-GGT cholestasis, ductal plate malformation, congenital hepatic fibrosis, and progressive portal hypertension[85,134,135].

Patients often present with hepatosplenomegaly, pruritus, and complications of portal hypertension, including gastrointestinal bleeding[85]. Disease progression is common, and many patients ultimately require liver transplantation[85,87]. Experimental data further support a causal role for *ZFYVE19* deficiency in hepatobiliary injury and fibrosis[90].

PFIC10 (MYO5B deficiency)

PFIC10 results from biallelic variants in *MYO5B*, which encodes myosin Vb, a motor protein involved in intracellular trafficking of apical membrane proteins[136,137]. The disease spectrum ranges from isolated cholestatic liver disease to microvillus inclusion disease or combined intestinal-hepatic phenotypes[75,95].

Although PFIC10 is classically associated with low-GGT cholestasis, biochemical variability has been observed, including cases with elevated GGT levels[136,138]. Clinical severity depends on the degree of intestinal involvement and residual protein function[95,137]. Preliminary clinical experience suggests that selected patients with *MYO5B* variants may benefit from odevixibat[139].

PFIC11 (SEMA7A deficiency)

PFIC11 is an extremely rare condition associated with variants in *SEMA7A*[115]. The limited available data describe a phenotype of cholestasis with elevated bile acids

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and relatively mild clinical features[115]. The biologic plausibility of this association is supported by broader evidence linking semaphorin 7A to fibrogenic and inflammatory signaling pathways[113,140]. Nevertheless, additional mechanistic and clinical studies are needed to establish the role of *SEMA7A* in PFIC[115].

PFIC12 (VPS33B deficiency)

PFIC12 represents a milder hepatic phenotype within the *VPS33B*-associated disease spectrum[141]. Patients present with low-GGT cholestasis and severe pruritus, often without the full features of ARC syndrome[141]. However, subtle extrahepatic findings, including renal, dermatologic, or skeletal abnormalities, may be present and require long-term follow-up[83,141]. Experimental studies further support a mechanistic role for *VPS33B* in cholestatic liver injury through disruption of epithelial polarity and trafficking pathways[79]. The broader phenotypic spectrum also includes ARKID syndrome, supporting marked genotype-dependent variability[77].

PFIC13 (PSKH1 deficiency)

PFIC13 is a recently described hepatorenal ciliopathy caused by biallelic *PSKH1* variants[120]. It is characterized by progressive cholestatic liver disease with frequent renal involvement, including structural abnormalities and glomerular injury[120].

Clinical features include hepatomegaly, splenomegaly, ascites, and progressive fibrosis, with some patients requiring liver transplantation. The presence of multisystem involvement supports its classification as a ciliopathy rather than an isolated hepatic disorder[120].

CLINICAL SPECTRUM AND NATURAL HISTORY

PFIC encompasses a broad and evolving clinical spectrum, ranging from severe neonatal cholestatic liver disease to milder or later-onset phenotypes presenting in adolescence or adulthood[1,2]. Although PFIC was historically regarded as a disorder of infancy, increasing use of next-generation sequencing has demonstrated

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that disease expression is highly variable and depends on the underlying molecular defect, residual protein function, and, in some cases, environmental or hormonal triggers[4,7]. As a result, PFIC is now better understood as a continuum of cholestatic disorders rather than a single, uniform pediatric entity[5].

Age at onset and mode of presentation

Most classical PFIC subtypes present during infancy or early childhood, often with persistent cholestasis, jaundice, pruritus, and poor weight gain[2,8]. Early-onset disease is particularly characteristic of PFIC1, PFIC2, PFIC4, PFIC5, PFIC6, PFIC10, PFIC12, and PFIC13. Among these, PFIC2 and PFIC5 are notable for especially early and aggressive onset, frequently in the neonatal period, with rapid progression to advanced liver disease[75,125]. In contrast, PFIC3 and PFIC7 may present later in childhood or adolescence, and adult-onset presentations are increasingly recognized in disorders related to *ABCB4*, *ABCB11*, *ATP8B1*, *TJP2*, and *USP53*[5,122,128]. These later presentations may manifest not as classical childhood PFIC, but as recurrent cholestasis, LPAC, ICP, drug-induced cholestasis, or otherwise unexplained chronic cholangiopathic liver disease[2,4].

Biochemical patterns: Low-GGT and high-GGT phenotypes

One of the most clinically useful frameworks in PFIC is the distinction between low- or normal-GGT cholestasis and high-GGT cholestasis[4,8]. PFIC1, PFIC2, PFIC4, PFIC5, PFIC10, and PFIC12 typically present with low- or normal-GGT cholestasis, reflecting defects in bile acid export, membrane stability, tight junction integrity, nuclear regulation, or intracellular trafficking rather than primary cholangiocyte injury[1,7]. PFIC7 and PFIC11 may also fall within this low-GGT spectrum, although the number of reported cases remains limited[115,128]. By contrast, PFIC3, PFIC8, and PFIC9 are more often associated with elevated GGT levels, consistent with cholangiocellular injury, ductular involvement, or abnormalities in bile duct morphogenesis[85,122,132]. This biochemical distinction is highly valuable in the diagnostic approach, although exceptions do occur, and overlap between categories should be expected in rare or complex phenotypes[4].

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Hepatic manifestations

Despite marked genetic heterogeneity, the core hepatic phenotype of PFIC includes cholestasis, jaundice, pruritus, hepatomegaly, and progressive fibrotic liver injury[1,2]. Pruritus is often one of the most debilitating features and may dominate the clinical picture even before advanced hepatic dysfunction becomes evident[13]. Progression from cholestasis to fibrosis, cirrhosis, portal hypertension, and liver failure is observed across multiple subtypes, although the tempo varies considerably[4,7]. PFIC2 and PFIC5 are among the most aggressive forms, often progressing rapidly to liver failure in infancy[75,125]. PFIC3 more commonly follows a fibrocholangiopathic course with biliary fibrosis and portal hypertension, whereas PFIC9 is particularly associated with ductal plate malformation[122], congenital hepatic fibrosis, and progressive portal hypertensive disease[85]. PFIC4 may range from relatively mild neonatal cholestasis to severe liver failure requiring transplantation[46,124]. Altogether, these observations indicate that the hepatic spectrum of PFIC is far broader than the classical image of isolated pediatric cholestasis.

Extrahepatic manifestations and multisystem involvement

A major driver of phenotypic diversity in PFIC is the extent of extrahepatic involvement. PFIC1 is the prototypical multisystem form, with diarrhea, pancreatitis or pancreatic insufficiency, hearing loss, and growth failure reflecting the broader epithelial expression of *ATP8B1*[45,121]. PFIC6 may also show a combined hepatointestinal phenotype, with chronic diarrhea, steatorrhea, and fat-soluble vitamin deficiency due to defective enterohepatic bile acid transport[2]. PFIC10 occupies a distinctive position because *MYO5B* deficiency may present with isolated cholestatic liver disease, severe microvillus inclusion disease, or a mixed intestinal-hepatic phenotype[95,137]. PFIC12, caused by *VPS33B* variants, may resemble an attenuated form of ARC syndrome, with subtle renal, musculoskeletal, or dermatologic manifestations in addition to cholestasis[141].

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PFIC13 has emerged as a clear hepatorenal ciliopathy, with renal agenesis, hydronephrosis, glomerular injury, and interstitial fibrosis accompanying progressive liver disease[120]. Even in genes classically considered liver-predominant, such as *TJP2* or *USP53*, extrahepatic findings including hearing abnormalities or developmental features have occasionally been reported[123,128]. These patterns emphasize that PFIC should not always be regarded as a purely hepatic disorder, but often as part of a broader epithelial, polarity, or ciliopathy spectrum.

Histopathology and clinicopathologic correlates

Histopathologic findings in PFIC vary substantially according to the underlying mechanism but frequently parallel the biochemical and clinical phenotype[4,7]. While PFIC1 classically presents bland cholestasis with progressive portal or periportal fibrosis over time, PFIC2 may show giant cell hepatitis, marked cholestasis, and reduced or absent BSEP expression on immunohistochemistry[1]. PFIC3 typically demonstrates biliary injury and progressive fibrosis consistent with chronic cholangiopathy[122]. PFIC4 is characterized by cholestasis and progressive fibrosis that may rapidly culminate in cirrhosis[123]. PFIC5 frequently shows markedly reduced or absent canalicular BSEP expression, reflecting the central role of FXR in regulating *ABCB11* transcription[125]. PFIC9 is particularly distinctive, with ductal plate malformation, congenital hepatic fibrosis, bile duct proliferation, ductular reaction, and, in some patients, features of sclerosing cholangiopathy[85]. These histologic differences are clinically useful because they reflect the underlying biology and may guide both diagnosis and prognosis.

Malignancy risk and long-term outcomes

Long-term outcome in PFIC is highly variable and strongly subtype-dependent[4,7]. Some patients experience relatively indolent disease or intermittent cholestasis, whereas others progress rapidly to liver failure in the first months of life. Native liver survival is particularly poor in severe *ABCB11*- and *NR1H4*-related disease[12,75]. In *ATP8B1* deficiency, fewer than half of affected individuals retain

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their native liver into adulthood[12]. Malignancy risk is another major determinant of prognosis. PFIC2 carries the strongest established association with early HCC, particularly in patients with severe genotypes and minimal residual BSEP function[1,4]. *TJP2*-related disease has also been linked to HCC in childhood, suggesting that chronic bile acid-mediated injury and severe epithelial barrier disruption may contribute to hepatocarcinogenesis[59]. In PFIC5, persistently elevated alpha-fetoprotein may complicate surveillance because it can reflect severe hepatocellular injury even in the absence of malignancy[75,126]. Overall, the natural history of PFIC is shaped by both hepatic progression and subtype-specific complications, reinforcing the need for long-term surveillance strategies tailored to molecular diagnosis[4].

Consistent with this clinical heterogeneity, in our institutional experience, a cohort of 25 patients with PFIC demonstrated a predominance of *ABCB4*-related disease (PFIC3, 40%), followed by *ABCB11* (PFIC2, 28%) and *TJP2* (PFIC4, 20%), while rarer subtypes, including *USP53* (PFIC7), *KIF12* (PFIC8), and *MYO5B* (PFIC10), were each identified in single patients. This distribution underscores both the relative frequency of major subtypes and the considerable genetic heterogeneity encountered in clinical practice. Notably, a patient with *USP53*-related disease (PFIC7) showed rapid resolution of refractory pruritus following odevixibat therapy, whereas a patient with *KIF12*-related disease (PFIC8) presented with chronic liver disease and pancreatitis in the absence of overt cholestasis, a presentation not well described in the literature.

Expanding adult phenotypes

Although PFIC has traditionally been conceptualized as a strictly pediatric disorder, compiling evidence firmly establishes a broader hepatological continuum that extends well into adulthood. This is particularly evident in disorders related to *ABCB4*, *ABCB11*, *ATP8B1*, *TJP2*, and *USP53*, in which milder, atypical, or intermittent phenotypes may first present beyond childhood[4,5]. Among these, *ABCB4*-related disease represents the most thoroughly mapped adult phenotypes, encompassing LPAC, ICP, and drug-induced or recurrent cholestasis[2,4]. Similarly,

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heterozygous or hypomorphic variants in *ABCB11* and *ATP8B1* have been associated with BRIC and episodic cholestatic phenotypes in adolescence or adulthood. Emerging data also indicate that variants in *TJP2* and *USP53* may underlie unexplained chronic cholangiopathic or cryptogenic liver disease in adults, further expanding the clinical spectrum beyond classical PFIC[4,5].

Consequently, adult manifestations may therefore encompass recurrent cholestasis, pruritus, LPAC, ICP, drug-induced cholestasis, and chronic fibrosing cholangiopathy[14]. These phenotypes are often underrecognized and may be misclassified as idiopathic liver disease unless genetic testing is considered. In this context, PFIC-associated genes should increasingly be viewed as contributors to cholestatic susceptibility across the lifespan, rather than being restricted to severe pediatric disease.

Long-term outcomes are likewise highly relevant in adulthood[28]. Patients with residual function may survive into adult life with their native liver but remain at risk for progressive fibrosis, portal hypertension, and disease exacerbation under hormonal (*e.g.*, pregnancy) or environmental triggers (*e.g.*, medications). In contrast, severe genotypes particularly those involving *ABCB11* or *NR1H4* are associated with early disease progression and limited native liver survival, often necessitating transplantation in childhood[14,28].

Malignancy risk is another critical consideration. HCC is well established in severe *ABCB11*-related disease, with cases reported even in early childhood, but the risk may extend into adolescence and adulthood in long-term survivors[14,20]. In addition, *TJP2*-related disease has been increasingly associated with pediatric and early-onset HCC, suggesting that disruption of epithelial barrier integrity and chronic bile acid-mediated injury may contribute to hepatocarcinogenesis[57-59]. Additionally, chronic cholangiopathic phenotypes, particularly those associated with *ABCB4* or ductal plate abnormalities, may also raise concern for biliary malignancies, including cholangiocarcinoma, although data remain limited[14,28,30].

Taken together, these comprehensive clinical observations highlight that PFIC represents a lifelong disorder with evolving clinical manifestations rather than a purely pediatric condition. These adult implications underscore the importance of

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long-term follow-up, early recognition of atypical phenotypes, and integration of genetic testing into adult hepatology practice, as well as the need for structured surveillance strategies tailored to molecular diagnosis.

DIAGNOSTIC APPROACH

The diagnostic evaluation of PFIC has evolved substantially with advances in molecular genetics. While PFIC was historically considered a diagnosis of exclusion, it is now increasingly approached as a genetically defined group of disorders in which early recognition and targeted testing play a central role. A structured diagnostic strategy integrating clinical presentation, biochemical profile, imaging, histopathology, and genetic analysis is essential for accurate classification and optimal management[4,7,142].

Initial clinical assessment

The diagnostic workup must initiate with a detailed clinical evaluation, encompassing the exact age at onset, family history, consanguinity, and the presence of extrahepatic features[4,7]. Early-onset cholestasis in infancy, particularly when accompanied by severe pruritus, growth failure, or fat-soluble vitamin deficiency, should raise strong suspicion for PFIC[2,7]. Furthermore, the recognition of extrahepatic manifestations may provide important diagnostic clues. For example, chronic diarrhea, pancreatitis, and hearing loss suggest *ATP8B1* deficiency, whereas renal or syndromic features may indicate *VPS33B*- or *PSKH1*-related disease[120,121,141]. A careful drug history and evaluation of perinatal factors are also necessary to exclude secondary causes of cholestasis[4].

Biochemical evaluation

Serum biochemical analysis remains a cornerstone of the diagnostic approach, with particular emphasis on GGT levels[4,8]. PFIC subtypes are broadly categorized into low- or normal-GGT cholestasis and high-GGT cholestasis, which provides an important initial framework for differential diagnosis[1,2]. Specifically, low- or normal-GGT cholestasis is characteristic of PFIC1, PFIC2, PFIC4, PFIC5, PFIC10, and

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PFIC12, and reflects defects in hepatocellular bile acid transport, membrane integrity, tight junctions, or intracellular trafficking[1,7]. In contrast, elevated GGT levels are more typical of PFIC3, PFIC8, and PFIC9, where cholangiocellular injury or bile duct abnormalities predominate[85,122,132].

Complementary laboratory findings may further support specific diagnoses. Elevated serum bile acid levels are a consistent feature across PFIC subtypes. Markedly elevated alpha-fetoprotein may be observed in PFIC2 and PFIC5, whereas coagulopathy disproportionate to liver function suggests *NR1H4* deficiency. Fat-soluble vitamin deficiencies and malabsorption may point toward PFIC1 or PFIC6[2,75,126].

Imaging studies

Abdominal ultrasonography serves as the indispensable, first-line imaging modality and is primarily used to exclude structural causes of cholestasis, such as biliary atresia, choledochal cysts, or obstructive lesions[4,8]. In PFIC, imaging findings are often nonspecific but may reveal hepatomegaly, splenomegaly, or features of portal hypertension[2].

In certain subtypes, more specific findings may be present. For example, PFIC9 may demonstrate features of congenital hepatic fibrosis or ductal plate malformation, while PFIC13 may show renal abnormalities such as increased echogenicity or structural anomalies[85,129]. Magnetic resonance cholangiopancreatography or cholangiography may be helpful in selected cases to further evaluate biliary anatomy[4].

Histopathological evaluation

Liver biopsy remains a valuable diagnostic tool, particularly when genetic testing is inconclusive or unavailable[4,7]. Histopathological findings may provide important clues regarding the underlying disease mechanism.

Bland cholestasis is commonly observed in PFIC1, whereas PFIC2 may show giant cell transformation and marked hepatocellular injury. PFIC3 is characterized by biliary injury and fibrosis, reflecting its cholangiopathic nature. PFIC4 often

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demonstrates progressive fibrosis, while PFIC9 may show ductal plate malformation and congenital hepatic fibrosis[45, 85,122,123].

Complementary immunohistochemical can further aid diagnosis. Reduced or absent BSEP expression supports PFIC2, whereas decreased MDR3 expression may indicate PFIC3. However, normal staining does not exclude disease, particularly in cases with residual protein function or trafficking defects[1].

Genetic testing

Genetic analysis has become the cornerstone of PFIC diagnosis and is now considered essential for definitive classification[4,142]. Genetic testing should be strongly considered in infants and children presenting with persistent cholestasis, particularly in the absence of biliary obstruction. Clinical and biochemical features that increase the likelihood of a genetic etiology include early-onset disease, low or normal GGT levels, severe pruritus, growth failure, and a family history of cholestatic liver disease or consanguinity.

Next-generation sequencing techniques, including targeted gene panels and whole-exome sequencing, enable the identification of pathogenic variants across the expanding PFIC spectrum[142]. Early implementation of these approaches is increasingly recommended, as it not only confirms the diagnosis but also reduces diagnostic delay, avoids unnecessary invasive procedures, and facilitates earlier initiation of mechanism-based therapies. In clinical practice, genetic testing is typically performed when initial clinical, biochemical, and imaging evaluations fail to identify a secondary cause of cholestasis.

Beyond establishing a definitive diagnosis, precise molecular characterization offers invaluable prognostic insights that directly shape therapeutic trajectories. For example, identification of truncating *ABCB11* variants may predict severe disease and limited response to medical therapy, whereas certain missense variants may be associated with residual function and a more favorable course[4,7].

Concurrently, genetic testing allows identification of heterozygous variants that may act as susceptibility factors in conditions such as ICP or drug-induced cholestasis, thereby expanding the clinical relevance of PFIC-associated genes

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beyond classical pediatric disease[4,5]. Taken together, these observations highlight the broader clinical implications of molecular findings.

Crucially, the evolving molecular classification of PFIC has direct implications for clinical decision-making. Mechanism-based stratification facilitates more targeted diagnostic evaluation such as prioritizing genetic testing based on characteristic biochemical profiles and informs therapeutic strategies. Furthermore, recognition of syndromic or extrahepatic involvement in disorders such as *ATP8B1*, *MYO5B*, or *VPS33B* deficiency can guide multidisciplinary evaluation and long-term management. Ultimately, integrating molecular mechanisms into clinical workflows is essential for personalized care in PFIC. To further illustrate the clinical implications of this framework, a mechanism-based functional stratification integrating variant type, clinical features, and therapeutic considerations is summarized in Table 2.

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DIFFERENTIAL DIAGNOSIS

The differential diagnosis of PFIC includes a wide range of neonatal and pediatric cholestatic disorders. These include biliary atresia, Alagille syndrome, bile acid synthesis defects and other metabolic liver diseases (such as alpha-1 antitrypsin deficiency and mitochondrial disorders), infectious causes, and endocrine abnormalities[4,8].

Differentiating PFIC from these conditions requires integration of clinical, biochemical, imaging, histological, and genetic data. The absence of biliary obstruction, presence of low- or high-GGT patterns, and identification of pathogenic variants are key elements in establishing the diagnosis[7,142].

TOWARD A MECHANISM-BASED DIAGNOSTIC ALGORITHM

With the expanding understanding of PFIC pathophysiology, diagnostic strategies are increasingly shifting toward a mechanism-based approach. Initial classification based on GGT levels, followed by targeted genetic testing, allows effectively constricts the differential landscape[4,5]. This approach also facilitates early identification of patients who may benefit from specific therapies, such as IBAT

inhibitors, and supports personalized management strategies based on genotype and predicted functional impact[5,7].

TREATMENT

The therapeutic landscape of PFIC has undergone a paradigm shift from predominantly supportive and surgical strategies toward targeted modulation of bile acid homeostasis. Current treatment approaches aim to reduce the intrahepatic bile acid pool, alleviate pruritus, delay disease progression, and minimize the need for liver transplantation. Concurrently, these strategies are uniquely personalized according to the underlying molecular mechanism and residual hepatocellular bile transport capacity[5,7].

Supportive and conventional pharmacologic therapy

Initial management remains centered on supportive measures, including optimization of nutrition and supplementation of fat-soluble vitamins. Cholestasis-associated malabsorption frequently results in deficiencies of vitamins A, D, E, and K, as well as impaired growth, necessitating early and sustained nutritional intervention[2,8].

Conventional pharmacologic therapies, including UDCA, rifampicin, cholestyramine, opioid antagonists, and antihistamines, constitute historical pillars of care, yet yield highly inconsistent and suboptimal therapeutic responses[7,11]. While UDCA may improve bile flow in certain subtypes such as PFIC3, its effect is generally insufficient in severe low-GGT PFIC (*e.g.*, PFIC1 and PFIC2)[2,7]. Similarly, rifampicin and bile acid sequestrants may provide partial relief of pruritus, but many patients remain refractory, underscoring the need for mechanism-based therapies[7,128].

Surgical interruption of enterohepatic circulation

Surgical biliary diversion has historically served as the primary disease-modifying intervention by reducing enterohepatic bile acid recirculation. Partial external biliary diversion has demonstrated long-term efficacy, with approximately 50% of patients

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achieving sustained reductions in serum bile acid levels over nearly a decade of follow-up. Early postoperative normalization of bile acids is strongly associated with improved native liver survival[11,12].

However, outcomes are highly dependent on genetic subtype. Patients with PFIC2 tend to respond more favorably, whereas those with PFIC1 frequently exhibit suboptimal responses, with long-term liver transplantation rates reaching 50%-75%. PFIC3 patients also show limited benefit, with approximately 60% progressing to transplantation[11,143]. Internal diversion procedures offer improved cosmetic outcomes but lack robust long-term data, and ileal bypass is associated with high relapse rates due to intestinal adaptation[11].

Despite its efficacy in selected patients, the invasive nature and morbidity associated with surgical diversion have limited its widespread applicability, particularly in the era of emerging pharmacologic alternatives[7].

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Liver transplantation

Liver transplantation remains the definitive treatment for advanced PFIC, particularly in patients with end-stage liver disease, refractory pruritus, or HCC[7,11]. Survival outcomes are favorable, with patient survival rates approaching 85% and graft survival around 77%[11]. Nevertheless, transplantation does not uniformly correct extrahepatic manifestations. In PFIC1, post-transplant complications such as chronic diarrhea, steatosis, and growth impairment frequently persist. In PFIC2, disease recurrence due to anti-BSEP antibodies has been reported, highlighting the complexity of disease biology[7,121]. These limitations emphasize the need for earlier and less invasive disease-modifying interventions.

IBAT inhibition: Targeting enterohepatic circulation

The introduction of IBAT inhibitors represents a major therapeutic advance. By blocking ileal bile acid reabsorption, these agents reduce the circulating bile acid pool and decrease hepatic exposure to toxic bile acids. This pharmacologic approach effectively mimics surgical biliary diversion while avoiding its invasiveness[9,10].

Odevixibat

Odevixibat is the most extensively studied IBAT inhibitor in PFIC. In the phase 3 PEDFIC-1 trial, involving pediatric patients with PFIC1 and PFIC2, odevixibat demonstrated significant improvements in both pruritus and biochemical parameters. Approximately 55% of treated patients achieved clinically meaningful pruritus reduction compared with 30% in the placebo group, while 33% achieved significant reductions in serum bile acid levels, compared with no responders in the placebo arm[9,10]. Therapeutic effects were observed as early as four weeks and sustained throughout treatment. Long-term extension studies confirmed durability of response, with additional benefits in sleep quality and growth parameters[10].

Real-world data further support these findings, demonstrating median reductions in serum bile acids of up to 80%-85%, along with pruritus response rates exceeding 70%. However, approximately one-quarter of patients remain non-responsive, and a subset of these ultimately require liver transplantation[144]. Notably, emerging evidence suggests that patients with PFIC7 (*USP53* deficiency) may also exhibit clinical and biochemical improvement with odevixibat therapy, further supporting the potential applicability of IBAT inhibition beyond classical PFIC subtypes[145].

Importantly, treatment response appears strongly influenced by baseline disease severity and genotype. Patients with very high baseline bile acid levels (> 300 μmol/L) have a significantly increased risk of non-response. Similarly, individuals with truncating *ABCB11* mutations and complete loss of BSEP function exhibit limited response, suggesting that residual bile secretion capacity is necessary for therapeutic efficacy[35,144].

Maralixibat

Maralixibat, another selective IBAT inhibitor, has been extensively studied in pediatric cholestatic disorders, particularly Alagille syndrome, and is increasingly being evaluated in PFIC. Clinical studies have demonstrated significant reductions in pruritus and serum bile acid levels, with sustained improvements over long-term follow-up[35].

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In PFIC cohorts, maralixibat has shown comparable efficacy to odevixibat in reducing pruritus, although data remain more limited. Importantly, maralixibat has demonstrated a clear exposure-response relationship, with higher doses associated with greater reductions in serum bile acids and pruritus scores[35].

Long-term studies indicate that maralixibat therapy may delay progression to surgical intervention or transplantation in selected patients. As with odevixibat, treatment response appears dependent on residual bile acid transport function, and patients with complete transporter deficiency exhibit reduced responsiveness[35].

Other IBAT inhibitors

Linerixibat and additional IBAT inhibitors are currently under investigation, primarily in adult cholestatic disorders such as primary biliary cholangitis and ICP. Although PFIC-specific data are limited these molecular agents further validate the concept that targeting enterohepatic bile acid circulation is a broadly applicable therapeutic strategy across cholestatic diseases[10,35].

Comparative efficacy and positioning of IBAT inhibitors

The emergence of IBAT inhibitors has shifted the treatment paradigm toward earlier pharmacologic intervention. Compared with surgical biliary diversion, IBAT inhibition offers a non-invasive alternative with comparable mechanistic effects. While surgical approaches achieve long-term bile acid normalization in approximately half of patients, IBAT inhibitors provide clinically meaningful pruritus reduction in greater than 50% and biochemical improvement in approximately one-third of patients in controlled trials, with higher response rates observed in real-world settings[9,11,144].

However, IBAT inhibitors are not universally effective. Patients with advanced disease, very high bile acid levels, or complete loss-of-function mutations remain at high risk of treatment failure. In such cases, early transition to surgical or transplant strategies may be warranted[35,144].

Safety profile

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The safety profile of IBAT inhibitors is highly favorable, with clinical trials demonstrating acceptable tolerability margins. The most common adverse events include diarrhea, abdominal discomfort, and mild elevations in liver enzymes. Diarrhea is dose-dependent but typically manageable. Fat-soluble vitamin deficiencies are frequently observed but may reflect underlying disease rather than drug toxicity, necessitating ongoing monitoring and supplementation[9,10].

Integration into a mechanism-based treatment algorithm

Accumulating evidence strongly supports a highly structured, mechanism-based therapeutic hierarchy. In patients with moderate disease and preserved bile transport function, IBAT inhibitors should be considered early, potentially delaying or avoiding surgical intervention. In contrast, patients with severe disease, high bile acid burden, or complete transporter deficiency may require early consideration of biliary diversion or transplantation[5,7,35].

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CONCLUSION

PFIC represents a genetically and mechanistically heterogeneous group of cholestatic liver disorders firmly establishing PFIC as a lifelong hepatological continuum rather than a strictly restricted pediatric entity. Advances in molecular genetics have shifted classification toward a mechanism-based framework, improving understanding of phenotypic variability and enabling more precise diagnosis. Accurate evaluation requires integration of clinical, biochemical, histopathological, and genetic data, while recognition of genotype-phenotype correlations and extrahepatic features is essential for prognostic assessment. Therapeutically, PFIC management is evolving toward mechanism-driven strategies. IBAT inhibitors have emerged as an important non-invasive option for selected patients, whereas surgical diversion and liver transplantation remain essential in advanced disease. Ultimately, PFIC exemplifies the transition toward precision medicine in hepatology, with ongoing advances expected to further refine diagnosis and improve long-term outcomes.

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Footnotes

Conflict-of-interest statement: The authors declare that they have no conflict of interest.

AI contribution statement: ChatGPT, Grammarly, and DeepL were used for English spelling and grammar correction. AI was used only for English language correction. Figure 1 was created with the aid of artificial intelligence. All AI-generated images were reviewed, verified, and approved by the authors, who take full responsibility for the accuracy, originality, and integrity of the final content.

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Figures and Tables

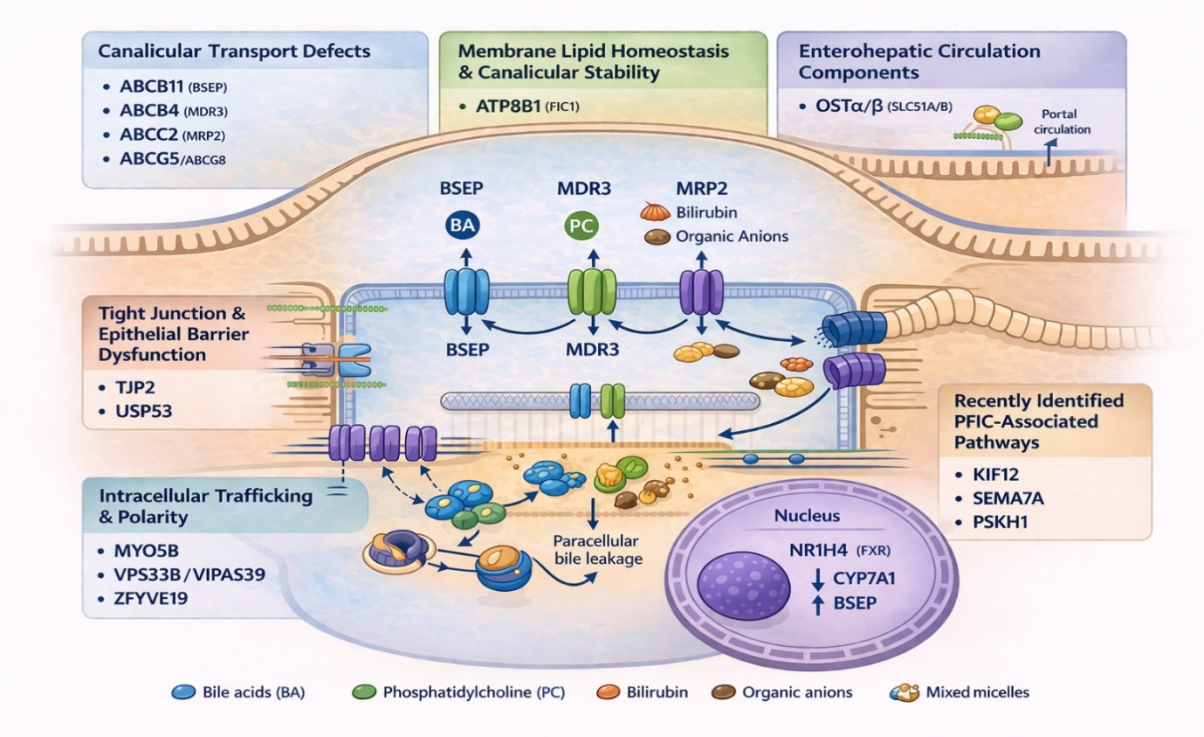


Figure 1 Mechanism-based classification of progressive familial intrahepatic cholestasis. This schematic illustrates the principal molecular pathways involved in hepatocellular bile formation and secretion and summarizes the major mechanistic categories underlying progressive familial intrahepatic cholestasis. Canalicular transport defects (*e.g.*, *ABCB11*, *ABCB4*, *ABCC2*, *ABCG5/ABCG8*) impair bile acid, phospholipid, and organic anion secretion, whereas defects in membrane lipid homeostasis (*ATP8B1*) compromise canalicular membrane stability. Tight junction abnormalities (*TJP2*, *USP53*) disrupt epithelial barrier integrity and promote paracellular bile leakage, while intracellular trafficking and polarity defects (*MYO5B*, *VPS33B*, *ZFYVE19*) impair canalicular transporter localization. Nuclear receptor dysfunction (*NR1H4*) alters bile acid synthesis and transporter regulation, whereas defects in enterohepatic circulation (*SLC51A/SLC51B*) disrupt bile acid recycling. OST: Organic solute transporter; BSEP: Bile salt export pump; MDR3: Multidrug resistance protein-3; MRP2: Multi-drug resistance protein 2; BA: Bile acid; PC: Phosphatidylcholine; FXR: Farnesoid X receptor; PFIC: Progressive familial intrahepatic cholestasis. The authors used artificial intelligence (AI) tools to assist in the generation of graphical content included in this manuscript. All AI-generated

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images were reviewed, verified, and approved by the authors, who take full responsibility for the accuracy, originality, and integrity of the final content.

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Table 1 Genes associated with progressive familial intrahepatic cholestasis and related cholestatic disorders

Gene (HGNC)	Locus	Mechanism	Protein	PFIC type/phenotype	OMIM	Inheritance
ABCB11	2q31.1	Canalicular bile salt transport defects	Bile salt export pump	Cholestasis, benign recurrent intrahepatic 2	605479	AR
				Cholestasis, progressive familial intrahepatic 2	601847	AR
ABCB4	7q21.12	Canalicular phospholipid salt transport defects	Multidrug resistance protein 3	Cholestasis, intrahepatic, of pregnancy 3	614972	AD, AR
				Cholestasis, progressive familial intrahepatic 3	602347	AR
				Gallbladder disease 1	600803	AD, AR
ATP8B1	18q21.31	Membrane lipid homeostasis/canalicular stability	Familial intrahepatic cholestasis protein 1	Cholestasis, benign recurrent intrahepatic	243300	AR
				Cholestasis, intrahepatic, of pregnancy 1	147480	AD
				Cholestasis, progressive familial intrahepatic 1	211600	AR
TJP2	9q21.11	Tight junction/epithelial barrier defects	Tight junction protein 2	Cholestasis, progressive familial intrahepatic 4	615878	AR

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				Hypercholanemia, familial 1	607748	AR
USP53	4q26	Tight junction/epithelial barrier defects	Ubiquitin-specific peptidase 53	Cholestasis, progressive familial intrahepatic, 7, with or without hearing loss	619658	AR
NR1H4	12q23.1	Transcriptional regulation of bile acid homeostasis	Nuclear receptor subfamily 1 group H member 4	Cholestasis, progressive familial intrahepatic, 5	617049	AR
MYO5B	18q21.1	Intracellular trafficking and epithelial defects	Myosin Vb	Cholestasis, progressive familial intrahepatic, 10	619868	AR
				Diarrhea 2, with microvillus atrophy, with or without cholestasis	251850	AR
VPS33B	15q26.1	Intracellular trafficking and epithelial defects	Vacuolar protein sorting-associated protein 33B	Arthrogryposis, renal dysfunction, and cholestasis 1	208085	AR
				Cholestasis, progressive familial intrahepatic, 12	620010	AR
				Keratoderma-ichthyosis-deafness syndrome, autosomal recessive	620009	AR
ZFYVE19	15q15.1	Ciliopathy/cholangiocyte	Zinc finger *FYVE*	Cholestasis, progressive	619849	AR

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		architecture defects	domain-containing protein 19	familial intrahepatic, 9		
SLC51A	3q29	Enterohepatic bile acid transport defects	Organic solute transporter alpha	Cholestasis, progressive familial intrahepatic, 6	619484	AR
KIF12	9q32	Ciliopathy/cholangiocyte polarity defects	Kinesin family member 12	Cholestasis, progressive familial intrahepatic, 8	619662	AR
SEMA7A	15q24.1	Signaling/inflammatory modulation	Semaphorin-7A	Cholestasis, progressive familial intrahepatic, 11	619874	AR
PSKH1	16q22.1	Ciliopathy/epithelial polarity defects	Protein serine kinase H1	Cholestasis, progressive familial intrahepatic, 13	620962	AR

HGNC: HUGO Gene Nomenclature Committee; OMIM: Online Mendelian Inheritance in Man; PFIC: Progressive familial intrahepatic cholestasis; AD: Autosomal dominant; AR: Autosomal recessive.

Table 2 Mechanism-based clinical classification of progressive familial intrahepatic cholestasis integrating variant type, phenotype, and therapeutic implications

	Residual function present	**Severe loss-of-function variants**
Typical variant type	Missense variants; partial loss of function; reduced but preserved protein activity	Nonsense variants; frameshift variants; canonical splice-site variants; biallelic null variants
Mechanistic contexts	Canalicular transport defects: Selected *ABCB11*, *ABCB4* variants. Membrane lipid homeostasis/canalicular	Canalicular transport defects: Truncating *ABCB11*, severe *ABCB4*. Membrane lipid homeostasis/canalicular stability:

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	stability: Selected *ATP8B1* variants. Tight junction/epithelial barrier dysfunction: Selected *TJP2*, *USP53* variants. Intracellular trafficking/polarity defects: selected *MYO5B* variants	Severe *ATP8B1*. Tight junction/epithelial barrier dysfunction: severe *TJP2*. Nuclear receptor/transcriptional regulation: *NR1H4*. Intracellular trafficking/polarity defects: Severe *MYO5B*, *VPS33B*
Genotype-phenotype correlation	Missense variants are often associated with residual protein activity. Genotype-phenotype correlation is evident in selected genes (*e.g.*, *ABCB11*, *ABCB4*). Clinical severity varies widely depending on residual function. Milder or later-onset phenotypes may be observed	Truncating or null variants are associated with absent protein function. Strong genotype-phenotype correlation with severe clinical presentation. Early-onset disease and rapid progression are common. Higher risk of complications (*e.g.*, liver failure, malignancy in selected subtypes)
Clinical implications	Variable severity. Later onset or slower progression may occur. Residual transporter activity may still permit bile flow	Early-onset, often neonatal or infantile. Rapid progression. Higher risk of advanced liver disease and complications
Therapeutic implications	IBAT inhibitors: More likely to be beneficial. UDCA: Especially relevant in *ABCB4*-related disease. Rifampicin/symptomatic therapy: May be useful for pruritus. PEBD/internal diversion: Can be considered in refractory cases with preserved native liver function	IBAT inhibitors: Limited or less predictable benefit. PEBD/internal diversion: Earlier consideration in progressive disease. Liver transplantation: Often required in severe or rapidly progressive cases. Multidisciplinary management: Especially important when extrahepatic involvement is present

Key determinant: Residual protein function is the primary driver of disease severity and therapeutic response across all PFIC

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mechanisms

PFIC: Progressive familial intrahepatic cholestasis; IBAT: Ileal bile acid transporter inhibitors; UDCA: Ursodeoxycholic acid; PEBD: Partial external biliary diversion.

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