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Progressive Familial Intrahepatic Cholestasis

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Authors
Bull, Laura N
Thompson, Richard J

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Progressive familial intrahepatic cholestasis

Laura N. Bull, Ph.D.*

Professor of Medicine

Department of Medicine and Institute for Human Genetics

University of California San Francisco

San Francisco, CA USA

Email: Laura.Bull@ucsf.edu

Phone: (415) 206-4807

Mailing address:

UCSF Liver Center Laboratory

Zuckerberg San Francisco General

1001 Potrero Avenue

Building 40, Room 4102

SF, CA 94110

*Corresponding author

Richard J. Thompson, M.D. Ph.D.

Professor of Molecular Hepatology

Institute of Liver Studies

King's College London

Mailing address:

Institute of Liver Studies

King's College Hospital

Denmark Hill

London

SE5 9RS

UK

Email: richard.j.thompson@kcl.ac.uk

Phone: +44 20 2399 4296

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Key words

Cholestasis, genetics, bile acids, pediatrics, PFIC

Key points

- Progressive familial intrahepatic cholestasis is an umbrella term and describes the severest form of a number of genetically discreet diseases
- Mechanisms of cholestasis include defects of canalicular transport, tight junction integrity, nuclear signaling, vesicular trafficking and membrane maintenance
- Human bile acids are highly detergent and the majority of cellular and organ damage in PFIC is mediated through a failure of bile acid and lipid homeostasis
- Genetic technology has helped reveal disease mechanisms, and can now be incorporated in new diagnostic algorithms

Synopsis

Genetic cholestasis has been dissected through genetic investigation. The major PFIC genes are now described. *ATP8B1* encodes FIC1, *ABCB11* encodes BSEP, *ABCB4* encodes MDR3, *TJP2* encodes TJP2, *NR1H4* encodes FXR and *MYO5B* encodes MYO5B. The full spectra of phenotypes associated with mutations in each gene are discussed, along with our understanding of the disease mechanisms. Differences in treatment response and targets for future treatment are emerging.

Introduction

Bile was recognized by the Ancient Greeks as one of the four humors; its importance is still recognized in 21st Century medicine, though maybe only by hepatologists. Bile formation is essential for normal liver and gastrointestinal functions. Jaundice is the most frequent manifestation of liver disease, and exemplifies the importance of bile in the disposal of a major waste product, bilirubin. Bile is in truth a complex liquid. It is an alkali solution, rich in a variety of lipids, containing numerous organic anions. Many of the latter are metabolites of drugs and other xenobiotics that have been conjugated and excreted by the liver. The lipids are largely composed of bile acids, which are themselves amphipaths with powerful detergent properties. Human bile acids are particularly powerful detergents. The biliary tree, like every other epithelium, is composed of cells, themselves defined by the lipid plasma membrane. The normal lipid composition of the canalicular and cholangiocyte apical membrane renders the cells more resistant to detergent damage than most epithelia. Furthermore, the detergent effect of bile acids is significantly moderated through packaging into mixed micelles, the other major component of which is phosphatidylcholine (PC).

The diseases described in this article are collectively known as progressive familial intrahepatic cholestasis, or PFIC. This term predates our understanding of the different disease mechanisms. It also fails to acknowledge that nearly all the genetic deficiencies that we will describe occur in spectra, ranging from infrequent symptoms, often precipitated by exogenous factors, through to severe early-onset disease. For most disease described, those with the most severe disease manifest autosomal recessive inheritance and could be labelled as having PFIC. The diseases described below represent defects in major processes involved in bile acid handling. (Bile acid synthesis defects are described elsewhere.) Bile salt

transport out of the liver is mediated by the bile salt export pump (BSEP) located in the canalicular membrane; BSEP expression is regulated by FXR. Correct localization of apical membrane transporters, such as BSEP, is dependent on intracellular trafficking processes, mediated in part by MYO5B. Inclusion of PC into bile is dependent on MDR3. FIC1 is important for distribution of lipids between the two leaflets of the apical membrane. The canaliculi themselves are sealed by tight junctions, themselves dependent on intracellular anchors, including TJP2. The major genes underlying the different types of PFIC, and typical phenotypes, are summarized in Table 1.

FIC1 deficiency

FIC1 (familial intrahepatic cholestasis 1), encoded by *ATP8B1*, is a member of the P4 family of P-type ATPases, ATP-dependent membrane transporters known as phospholipid 'flippases'[1]. Flippases translocate phospholipids in from the external, to the cytoplasmic, membrane leaflet. Floppases move phospholipids out, in the opposite direction. Although the identification of *ATP8B1* as a cholestasis gene occurred early in the focused application of genetics to hereditary cholestasis [2], it has been challenging to determine the precise mechanism(s) whereby FIC1 deficiency results in cholestasis.

FIC1 is expressed in a variety of tissues, including the liver and intestine [2,3]. Some early data suggested FIC1 transports phosphatidylserine, but accumulating data more strongly support PC as a preferred substrate[4-6]. When FIC1 is not available to help maintain normal distribution of lipids between the two membranes of the lipid bilayer, the canalicular membrane may become vulnerable to damage by bile acids in the canaliculus [7]. Proteins in this membrane, including BSEP, may also have impaired function [7]; such impaired BSEP function would be expected to contribute to cholestasis. Through its phospholipid flippase

function, FIC1 may also play a role in membrane trafficking and vesicular transport, functions which if impaired, could contribute to cholestasis [1]. Some studies suggest that loss of FIC1 impairs FXR signaling, although other studies do not support that mechanism[8-13]. FIC1 may also play a role in the innate immune response, attenuating the inflammatory response, perhaps through a role in endocytosis [14].

Autosomal recessive mutations in *ATP8B1* can result in cholestatic disease along a continuum of severity, with PFIC typically diagnosed in patients with likely complete loss of FIC1 function[2]. Patients with milder phenotypes, including episodic cholestasis, have also been found to harbor mutations in *ATP8B1*. Such patients have been labelled as BRIC, or benign recurrent intrahepatic cholestasis. It is important to realize that lateness of onset does not preclude disease progression [15].

In PFIC due to FIC1 deficiency, patients typically present with jaundice within the first few months of life. At or near presentation, patients may also have clinically significant diarrhea. Biochemically, patients have hyperbilirubinemia, normal serum γGT activities, high serum bile acids, and mildly elevated transaminase activities. Consistent with the broad tissue distribution of FIC1, patients often have extrahepatic manifestations during the course of disease, such as diarrhea, pneumonia, hearing loss, pancreatic disease, resistance to parathyroid hormone, and growth impairment beyond that attributable to cholestasis [2,16-19].

Early in disease course, histologic findings are of bland intracanalicular cholestasis, without signs of significant hepatocyte injury. As disease progresses, inflammation, fibrosis, bile duct proliferation and cirrhosis may become apparent. Ultrastructural evaluation using transmission electron microscopy may demonstrate coarsely granular bile in the canaliculus [20]. Immunostaining for FIC1 has not been established for routine clinical use. However, surrogate markers of FIC1 deficiency are fairly widely used. In particular, reduced canalicular

staining for ectoenzymes such as γGT, CD10, and CEA is typical. Staining for membrane transporter proteins including BSEP and MDR3 is maintained[21].

Disease is often managed with medication or non-transplant surgical interventions such as partial external biliary diversion [17,22]. When end-stage liver disease occurs, liver transplantation is required for survival. After liver transplantation, FIC1 patients usually develop marked graft steatosis, and inflammation [17,22,23]. The liver graft abnormalities can be greatly improved by creating a total external biliary diversion, which many would now perform at the time of transplant [24-26]. Many patients also suffer diarrhea, which can be worse than before the transplant [17,22,24]. Given FIC1's roles in many tissues, and these post-transplant manifestations, liver transplant isn't curative of FIC1 deficiency.

BSEP deficiency

BSEP is encoded by *ABCB11* [27]. Severe BSEP deficiency is the most frequently seen form of PFIC. At any moment in time there is either enough bile acid-transporting capacity, in which case there is no accumulation of bile acids within the hepatocyte, or there is not enough, and bile acids do accumulate and disease ensues. Patients that have a very significant reduction in BSEP function, and hence PFIC, generally present in the first few months of life, and manifest jaundice, high serum bile acids and transaminases, normal serum γGT levels, fat soluble vitamin deficiency and, in due course, pruritus [16,17]. Histology of the liver, at this stage, shows marked intracellular cholestasis and, usually, obvious giant cell transformation. BSEP antibody staining is widely available, and is abnormal or absent in >90% of severe cases [28].

Individuals with later-onset disease caused by biallelic mutations in *ABCB11* have been identified, presenting as late as the third decade [15,29,30]. Some have gone on to need liver transplantation, despite presenting after infancy.

Cholestasis patients can have various milder phenotypes caused by mutations in one or both copies of this gene [29]. Patients with reduced transport capacity may have adequate BSEP function most of the time. A true pathogenic mutation may only be present on one allele, although polymorphisms (such as p.V444A) influencing levels of protein expression or function on the other allele probably mean that such individuals have reduced function on both alleles [31]. Somewhere nearer 25% of ideal BSEP function is probably the threshold for patients at risk of cholestasis, be that induced by drugs, pregnancy, viruses, malignancy or less obvious precipitants.

Two mutations, relatively common amongst European BSEP deficiency patients, appear to result in some residual function[32]. Patients with at least one copy of either of these mutations (p.E297G or p.D482G) can present with either PFIC or a less severe phenotype[15,29,30]. They have also been shown to have better outcomes, and improved responses to some treatments, compared to other patients with early-onset BSEP deficiency[17,22,33].

Most drugs treatments, to date, have not shown significant impact on the disease in severe BSEP deficiency patients. Ursodeoxycholic acid (UDCA) is widely prescribed, and in a few cases a significant improvement has been described[17]. Partial external biliary diversion (PEBD) helps a proportion of patients[17,22,33]. PEBD relies on the presence of bile acids in bile and has shown a better response in those shown to have some residual BSEP function. In particular, good response has been seen in 60% of individuals with at least one copy of either of the variants p.E297G or p.D482G[22].

Missense mutations are typically annotated using the predicted change of amino acid. This could be taken to infer that it is the change in amino acid that causes the loss of function. We know, however, that many such mutations actually have their effect at an RNA level[34]. Many others do not so much interfere with transport function, for instance, but actually reduce the amount of protein getting to the correct cellular location. Incorrectly localized apical proteins, such as BSEP and indeed CFTR, are potential targets for drug treatment[35,36]. A number of small molecules have been tested in both conditions as chaperones[37,38], and in the case of CFTR, are now licensed. This is certainly an area for further laboratory and clinical research.

BSEP deficiency, manifesting as PFIC, typically progresses to end-stage liver disease within a few years. Many patients have actually been transplanted due to severe pruritus before they reach end-stage disease. Despite the early use of transplantation, as many as 15% have developed hepatocellular carcinoma, either clinically or at explant[39]. Most of the cancers have occurred by 5 years of age. Transplantation is therefore widely and successfully used to treat severe BSEP deficiency. As the protein is only expressed in the liver, transplantation should be an excellent treatment. However, an unusual complication has become apparent. Some patients have been noted to have recurrence of exactly the symptoms that they had pre-transplant[40,41]. It transpires that this is secondary to allo-reactive antibodies, specific to one extracellular loop of the protein, which block the function of the normal protein in the transplanted liver[42]. Most patients with this condition have responded to modest increases in immunosuppression. Some patients have proved to have resistant disease and have required B-cell depletion for a number of years until they have regained control with conventional immunosuppression[43,44].

MDR3 deficiency

MDR3 is encoded by *ABCB4*. Perhaps the disease caused by mutations in this gene should not be called PFIC at all, as it is really a cholangiopathy[45]. MDR3 transports PC from the inner to the outer leaflet of the canalicular membrane, where it is then available for incorporation into bile micelles[46]. Deficiency of PC in bile means that there are free bile acids, resulting in a detergent bile, injurious to cholangiocyte membranes. Unlike BSEP deficiency, the primary defect in MDR3 deficiency does not cause retention of bile acids in the hepatocyte, and therefore does not directly cause cholestasis. Symptoms only occur as a consequence of the damage resulting from the ensuing cholangiopathy[47]. Even complete deficiency of MDR3 can take several years before presenting clinically[48]. At presentation, the serum γGT level is usually markedly elevated, and histology shows cholangiolytic changes, and occasionally, clefts where cholesterol crystals have been. Immunohistochemical staining for MDR3 is not quantitative and is only negative in the complete absence of protein[49].

A partial loss of MDR3 function will lead to a more slowly progressive disease; there is a very wide range of diseases, manifestations and ages of presentation[48,49]. Unfortunately, 50% function, as seen in heterozygotes for complete loss of function alleles, is sufficient impairment for damage to occur in some individuals[48]. If investigated, such individuals may have evidence of disease in the first few decades; however, clinical presentation may only be with end-stage disease, or hepatobiliary malignancy. Heterozygous relatives of MDR3 deficiency patients are therefore at increased risk of slowly progressive disease. Many patients have also been described without intrinsic cholangiopathy, but with a reduction in biliary PC such that they are highly predisposed to cholesterol precipitation and stone formation[50]. Such individuals can develop extensive intrahepatic cholelithiasis and be extremely difficult to manage.

The cholangiopathy in MDR3 deficiency is caused by biliary bile acids. Reduction of the bile acid pool size, via PEBD, has been rarely attempted and has probably never been undertaken early enough in the disease process to prevent progression. Instead, a reduction in the detergent nature of the bile has been achieved through supplementation with UDCA[49]. Enrichment of the bile salt pool by UDCA is probably limited by its inability to suppress the synthesis of endogenous bile salts, via FXR[51]. In milder forms of MDR3 deficiency, UDCA frequently achieves very significant biochemical improvement. It is not yet clear if this results in an improvement in long-term clinical outcomes. Treatment of severe MDR3 deficiency by all current modalities, short of transplantation, has probably been frustrated by the relatively late diagnosis. It is, however, a disease which may well respond to the use of highly hydrophilic bile acids, FXR agonists and endogenous bile salt depletion, possibly in combination. Transplantation is a very effective treatment of end-stage MDR3 deficiency, with one proviso. The clinical manifestations described above, notably in heterozygotes, do mean that many first-degree relatives do not make good living-related donors. Where there are no other options, UDCA should certainly be maintained, and close surveillance observed.

TJP2 deficiency

Most of the conditions described here have been identified in their severest forms first. TJP2 deficiency is an exception. Homozygosity for a particular missense change manifests as hypercholanemia amongst the Amish, with reduced penetrance [52]. These patients did not manifest chronic liver disease. On the other hand, severe progressive liver disease was later attributed to biallelic mutations predicted to cause complete loss of TJP2 function [53]. Patients

with intermediate loss of function have subsequently been identified (unpublished data, the authors).

The tight junction proteins (1, 2 and 3) are not part of the tight junction itself, but are cytoplasmic. They are closely associated with the proteins that do form tight junctions, such as the claudins. Deficiency of Claudin-1 has been described, associated with a cholangiopathy, as might be expected [54-56]. By contrast, deficiency of TJP2 is associated with cholestasis, but not a cholangiopathy, suggesting that the tight junction barrier function is not badly disrupted. The disease mechanism is not known, but tight junctions have a number of other functions. They form a selective barrier, allowing passage of some molecules and not others. They also form a fence between the basolateral and apical membranes; these two membranes differ markedly in both protein and lipid composition. Lastly, TJP2 has been shown to have a quite separate function, travelling to the nucleus, where it is transcriptionally active and inhibits cell cycle progression.

The patients so far described with complete loss of function of TJP2 have all had early-onset progressive liver disease, most requiring liver transplantation with the first few years of life. At presentation, these patients have had near-normal levels of γGT. Several cases have developed extra-hepatic disease; respiratory and neurological most frequently [53] (and authors unpublished data). This is consistent with the widespread expression of TJP2, and the general importance of tight junctions. Histology has shown rather non-specific features, with intracellular cholestasis and scant giant cells early in disease course, though immunohistochemical staining for TJP2 itself has been useful in identifying cases. Patients with TJP2 deficiency and HCC have been described[57,58].

FXR deficiency

The farnesoid X receptor (FXR), encoded by *NR1H4*, is a nuclear receptor and transcription factor, for which bile acids are the natural ligand. Along with a wider role in metabolic regulation, it plays an important role in bile acid homeostasis [59-61]. If hepatic bile acid levels are high, FXR represses bile acid synthesis and uptake, and increases export of bile acids out of the hepatocyte. In intestinal cells, in response to bile acids, FXR induces expression of FGF19, which then travels to the liver, where it represses bile acid synthesis. FXR is involved in regulation of other known cholestasis genes, including *ABCB11* and to a lesser degree, *ABCB4* [62,63]. Based on the understood roles of FXR, it has been anticipated that complete loss of function of FXR would result in severe cholestasis and liver damage; the first, and only, cases of PFIC attributable to autosomal recessive FXR mutation reported to date are those of two pairs of affected sibling [64]. FXR deficiency thus appears to be a relatively rare cause of PFIC.

One pair of affected siblings was homozygous for a mutation that prematurely truncates the protein in the FXR's DNA binding domain (p.R176*). The other pair of affected siblings was compound heterozygous for an in-frame insertion into the DNA binding domain, and a large deletion, which eliminated the 1st two coding exons of FXR. Evidence in all patients indicated loss of FXR protein expression and function.

Three of the patients presented with cholestasis by 7 weeks of age, while the fourth presented at birth with ascites, pleural effusions, and intraventricular hemorrhage; the latter patient died of an aortic thrombus at age 5 weeks. At initial biochemical evaluation, patients demonstrated conjugated hyperbilirubinemia, substantially elevated serum transaminases, and γGT activities normal for age. Serum bile acids were measured in only one patient, and were elevated. Coagulation was also impaired. Characterization of the coagulopathy determined that these patients had severe vitamin K-independent coagulopathy with early

onset, occurring well before end-stage liver failure. This finding is consistent with evidence for a role of FXR in regulation of coagulation [64,65]. Alpha-fetoprotein (AFP) was assayed in three patients and found very elevated in two. Early-onset vitamin K-independent coagulopathy and markedly elevated AFP levels may help to distinguish FXR deficiency from other genetic forms of PFIC. In liver of FXR deficiency patients, intralobular cholestasis was seen, along with ductular reaction, giant cell transformation, and hepatocyte ballooning. Fibrosis, infiltration by inflammatory cells, and cirrhosis appeared as disease progressed. Immunohistochemical studies found expression of BSEP and FXR undetectable in all 4 patients, consistent with FXR's important role in inducing expression of BSEP, while MDR3 was detected.

The three patients presenting with cholestasis had particularly rapid progression to end-stage liver disease, in comparison with other genetically understood forms of PFIC. Two underwent liver transplantation (at ages 22 months and 4 months), while the 3rd died at 8 months of age, while awaiting transplant. After liver transplantation, serum γGT remained normal, but ALT was sometimes elevated, and one patient developed mild conjugated hyperbilirubinemia. These findings were accompanied by histologically diagnosed hepatic steatosis, reminiscent of that seen in many patients with FIC1 deficiency after liver transplantation. The steatosis and mild biochemical abnormalities seen in FXR deficiency after liver transplant may be a consequence of lack of induction of FGF19 by intestinal FXR.

MYO5B deficiency

Myosin 5B (MYO5B) plays a role in plasma membrane recycling, transcytosis, and epithelial cell polarization in multiple tissues, including enterocytes and respiratory epithelial cells, as well as hepatocytes [66-70]. In hepatocytes, MYO5B interacts with RAB11A to facilitate

normal trafficking of ABC transporter proteins, including BSEP, to the canalicular membrane [71,72]. Autosomal recessive mutations in *MYO5B* were initially identified in a proportion of patients with microvillus inclusion disease (MVID) [68,73]. A subset of MVID patients with *MYO5B* mutations developed cholestasis as well [71]. Most recently, mutations in *MYO5B* have been reported in some patients diagnosed with cholestasis, in the absence of obvious features of MVID [74,75].

Fifteen patients bearing autosomal recessive *MYO5B* mutations, with cholestasis but without a MVID diagnosis, have been reported [74,75]. Some of these patients have manifested chronic cholestasis, although typically without rapid progression, while others have had recurrent bouts of cholestasis, or a single transient bout of cholestasis to date. Comparison of mutation profiles in MYO5B deficiency patients with MVID diagnosis, versus those with isolated cholestasis diagnosis, suggests that patients with MVID are more likely to have biallelic severe mutations, and biallelic mutations affecting the MYO5B-RAB11A interaction domain [75]. Thus, milder MYO5B deficiency may tend more often to manifest as cholestasis in absence of notable intestinal disease.

In cholestasis due to MYO5B deficiency, patients typically present within the first 2 years of life, with jaundice, pruritus and hepatomegaly. Conjugated hyperbilirubinemia is accompanied by high serum bile salts, low-to-normal serum γGT activity and mild-to-moderately elevated transaminases.

Histological evaluation revealed giant cell change, and hepatocellular and canalicular cholestasis; fibrosis was sometimes present. Inmmunohistochemical studies revealed that MYO5B and RAB11A in these patients typically demonstrate abnormal staining, with reduced canalicular staining and increased cytoplasmic staining, the latter showing a granular appearance. BSEP and MDR3 staining also was abnormal, with the suggestion of

patchy subcanalicular staining; in other patients, BSEP was not detected or staining was weak.

In most MYO5B deficiency patients with isolated cholestasis, their condition was managed with medications, including UCDA, and/or rifampicin, cholestyramine, or traditional Chinese medicine. However, one patient underwent PEBD, and another died aged 2.6 years, whilst awaiting liver transplantation.

It is likely that MYO5B deficiency results in cholestasis at least partially through decreased targeting of BSEP to the canalicular membrane; the cell membrane is likely also dysfunctional in other ways.

Genetics

Genetics has allowed the discovery and definition of the diseases described above. For many years, diagnosis has relied on assembly of clinical features, often supported by liver histology and immunohistochemistry data. It was often then possible to match patients with the original published descriptions and confirm the diagnosis by Sanger sequencing of the appropriate gene.

In the recent years, genetic technology has developed such that a quite different diagnostic algorithm is possible. Next generation sequencing technology makes it possible to sequence multiple genes, in multiple individuals, simultaneously. Its most comprehensive form is whole genome sequencing (WGS). Whole exome sequencing (WES) restricts sequencing to the exons of most genes. WES is a widely used research technique, responsible for the identification of some of the genes in this article. Over the next few years, WES will become a routine diagnostic tool, followed by WGS[76]. In 2018, the most widely used strategy is targeted panels of genes. For cholestatic liver disease, such panels

typically include all the PFIC-related genes, genes underlying Alagille syndrome, Arthrogyposis, Renal dysfunction and Cholestasis syndrome and Dubin-Johnson syndrome, those causative of Niemann-Pick disease and the genes underlying the inborn errors bile acid synthesis. Such a panel can now be used much earlier in a diagnostic pathway, perhaps as soon as biliary atresia and structural defects have been excluded. Biochemical and other phenotype-based testing for some of the above list can now be avoided, or perhaps used only selectively to confirm the genetic diagnosis.

One consequence of using genetics in this way is that we are learning that not everyone with a given genetic etiology has the same phenotype as was originally described. This is most notably the case for patients with partial Alagille syndrome, but it has also become clear that FIC1 and MDR3 deficiencies both have very broad ranges of presentation, and there are clearly patients with mutations in ABCC2 that do not have a Dubin-Johnson phenotype. A minority of PFIC cases elude genetic diagnosis using current methods. Some probably have mutations in the known PFIC genes which are not detectable by routine clinical testing, and others will have mutations in genes not currently implicated in PFIC.

Conclusions

Progressive familial intrahepatic cholestasis is an ever-growing family of diseases. Genetics has helped unravel the disease mechanisms, and we can better understand the spectra of phenotypes associated with mutations in each gene. Different responses to medical and surgical management, based on genetic etiology and molecular mechanisms, are becoming apparent. A precision medicine approach to treatment is now realistic. Current genetic technology is greatly speeding diagnoses and improving our understanding of the full impact of mutations in these genes in children and adults.

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Deficiency	Mutated gene	Typical clinical characteristics	Characteristic histology at diagnosis	Typical clinical outcomes
FIC1	*ATP8B1*	Multisystem disease Normal γGT Only modest elevation of transaminases	Bland canalicular cholestasis Coarsely granular canalicular bile	Moderate rate of progression Post-transplant hepatic steatosis and diarrhea
BSEP	*ABCB11*	Normal γGT High risk of HCC High incidence of gallstones	Giant cell transformation	Moderate to rapid progression Allo-antibody formation after transplant in some
MDR3	*ABCB4*	Progressive cholangiopathy Elevated γGT	Cholangiolytic changes	Highly variable rate of progression
TJP2	*TJP2*	Some extra hepatic features Near normal γGT	Bland cholestasis	Rapid progression
FXR	*NR1H4*	Early onset coagulopathy Normal γGT Markedly elevated AFP	Intralobular cholestasis Ductular reaction Giant cell transformation	Very rapid progression Post-transplant hepatic steatosis
MYO5B	*MYO5B*	Normal γGT Variable degree of intestinal involvement	Giant cell change Hepatocellular and canalicular cholestasis	Slow progression

Table 1. Summary of the typical features of progressive familial intrahepatic cholestasis associated with different genetic etiologies.

γ-glutamyltranspeptidase (γGT); hepatocellular carcinoma (HCC); α-fetoprotein (AFP)