REVIEW ARTICLE

Progressive familial intrahepatic cholestasis

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INTRODUCTION

Progressive familial intrahepatic cholestasis (PFIC) is a term to denote a heterogenous group of autosomal recessive disorders of biliary transporters with varied spectrum of hepatic manifestations ranging from mild liver disease, isolated pruritus affecting quality of life, recurrent cholestasis, gall-stones, intrahepatic cholestasis of pregnancy to neonatal cholestasis, infantile liver failure, portal hypertension, growth failure, advance end-stage liver disease necessitating liver transplantation (LT) [1-3]. The first three of these disorders (types 1, 2 and 3) have been numbered based on their discovery. The estimated prevalence in the western world is around 1:18000 with PFIC comprising 9-13% of diagnosis among children with intrahepatic cholestasis or liver disease. Overall, PFIC2 (BSEP deficiency) is the commonest (21-91%) followed by PFIC1 (FIC1 deficiency) (30-41%) [4-8]. With advancement in genetic technology newer entities like mutations in tight junction protein-2 (TJP2), farnesoid-X receptor (FXR) and Myosin-5B (MYO5B) have come into picture [1, 9-11]. Hence, the better terminology for each one of them is by the name of the defective transport or structural protein or the gene involved. The current text discusses the pathophysiological basis, clinical characterization, diagnosis, histological features, and management strategies for these disorders. Table 1 presents the differentiating features of different types of PFIC, their clinical course and outcome.

BILIARY TRANSPORT AND REGULATION

Bile constitutes bile acids, phospholipids, conjugated bilirubin, cholesterol, heavy metals and several different detoxified and modified metabolites. Bile acids are the main components of bile, and it is the flux/recirculation of bile acids that is the main driving force to bile formation. Hepatocyte polarity is primarily responsible for the synthesis and transport of bile acids. Bile acids are synthesized in hepatocytes either via neutral (classical) pathway or acidic (alternative) pathway. These bile acids are then conjugated to either glycine or taurine and become bile salts which are negatively charged at physiological pH. The basolateral hepatocyte membrane (sinusoidal membrane), is responsible for the uptake of conjugated bile salts via sodium dependent bile salt transporter (NTCP, gene *SLC10A1*). The multispecific organic anion transporting polypeptides like OATP-A and OATP-C, and to some extent OATP8 are involved in sodium independent bile salt uptake. Bile acids in the form of bile salts are then excreted out of the hepatocyte through canalicular membrane via bile salt exporter pump (BSEP), an ATP-binding cassette (ABC) transporter which is encoded by ABCB11. Hepatocyte excretion of phospholipids is mediated by multidrug resistance protein 3 (MDR3) encoded by ABCB4 gene [12, 13]. This protein (MDR3) acts as a floppase, which translocates phospholipids from the inner to the outer leaflet of the lipid bilayer of the canalicular membrane. Familial intrahepatic cholestasis 1 (FIC1) protein, encoded by ATP8B1 gene, is also expressed on canalicular membrane and it helps in bile salt transport by maintaining the enrichment of aminophospholipids on the inner leaflet of the canalicular membrane (flippase). In most eukaryotic cells, phosphatidylcholine and sphingolipids are concentrated in the exoplasmic leaflet, whereas the aminophospholipids (phosphatidylserine and phosphatidylethanolamine) are largely confined to the cytoplasmic leaflet. FIC1 protein thus helps in maintaining this asymmetrical gradient (Figure 1) [14].

Expression of ABCB11 and ABCB4 and other transporters is regulated by farnesoid-X receptor (FXR) protein, which is a nuclear receptor and transcription factor and a natural ligand for bile acids. FXR binds as a heterodimer with the retinoid X

receptor (RXR) which then exerts its actions. FXR can also downregulate the transcription of specific target genes indirectly via another nuclear receptor, the small heterodimer partner (SHP). FXR plays an important role in bile acid homeostasis. With high hepatic bile acid levels, FXR represses bile acid synthesis and uptake, and increases their export out of the hepatocytes. In the mucosa cells of the ileum, bile acids bind to FXR leading to activation of the transcription of fibroblast growth factor (FGF19) and subsequently FGF19 is secreted into the portal circulation. At the hepatocyte surface, FGF19 binds to FGFR4/bKlotho leading to activation of transcription of short heterodimer partner (SHP). This complex interaction of FGFR4/bKlotho and FXR-SHP block bile acid synthesis by blocking the transcription of CYP7A1 enzyme which is mediated by liver receptor homologue-1 (LRH-1) and hepatocyte nuclear factor-4*a* (HNF4*a*). CYP7A1 is the rate-limiting enzyme in the synthesis of bile acids from cholesterol. The FXR-RXR complex directly induces the expression of organic solute transporters (OST) *a* and ß (in ileal enterocytes and in the basolateral membrane of hepatocytes) as well as intestinal expression of the intestinal bile acid-binding protein (IBABP). In addition to directly activating the main BS efflux systems, under cholestatic conditions, FXR concurrently downregulates the main BS uptake systems, primarily NTCP in the basolateral membrane of hepatocytes and apical sodium-dependent bile acid transporter (ASBT, gene *SLC10A2*) in the ileal epithelium (Figure 1) [14, 15].

Intracellular trafficking of the transporters including BSEP and localization to the canalicular membrane is regulated by myosin-5B (MYO5B)/RAB11A recycling endosome pathway [1, 10]. Finally, there are tight junction proteins (TJP 1, 2, 3) which are cytoplasmic proteins and not part of tight junction itself, but closely associated with other proteins called claudins, which form tight junctions (Figure 2) [1, 16].

Mechanism of Pruritus in PFIC:

As pruritus is the dominant manifestations of most PFICs, it is important to understand its pathogenesis. Mechano-insensitive C-nociceptors in the skin with unmyelinated nerve endings are sensitive to itching. These C-fibers play role in transmitting the signals from skin to dorsal route ganglion from where it goes to ventromedial nucleus of thalamus via spinothalamic tract. From thalamus itch signals reach to the primary sensory cortex, inferior parietal lobe and anterior cingulate gyrus. Both pain and itch fibers involve the activity of TRPV1 (capsaicin receptor). TRPV1 is directly activated by capsaicin (red hot chilli pepper), high temperature (>43^{0}C), low pH (<5.9) and by lysophosphatidic acid (LPA). LPA is an important mediator of cholestatic itch. Autotaxin is an enzyme which converts lysophosphatidylcholine into LPA and is a useful marker of cholestasis and pruritus. Neurotransmitters involved in the transmission of itch sensation are Natriuretic polypeptide b (Nppb) and Gastrin releasing peptide (GRP). Various pruritogens involved in the pathogenesis are histamine, bile salts, serotonin, LPA, endogenous opioids, progesterone and estrogen, and have implication in specific targeted therapies against pruritus [17].

FIC1 DEFICIENCY (BYLER'S DISEASE, PFIC1)

FIC1 protein is a member of the P4 family of P-type ATPases, ATP-dependent membrane transporters known as phospholipid "flippases". FIC1 is expressed in a variety of tissues, including liver, intestine, pancreas and kidneys [18]. When FIC1 is not available to help maintain normal distribution of lipids between the 2 membranes of the lipid bilayer, the canalicular membrane may become vulnerable to bile canaliculus. Proteins in this membrane, including BSEP, also may have impaired function contributing to cholestasis. It has been proposed that FIC1 also plays a role in membrane trafficking and vesicular transport. FIC1 may also play a role in the innate immune response, attenuating the inflammatory response, perhaps through a role in endocytosis [19, 20].

Genotype-phenotype correlation: Genotype-phenotype associations are complex with ATP8B1 mutations. The disease may represent a continuum of severity, with PFIC typically diagnosed in patients with likely complete loss of FIC1 function due to nonsense, frameshift and large deletion mutations. Patients with milder phenotypes, including episodic cholestasis like benign recurrent intrahepatic cholestasis 1 (BRIC1), transient neonatal cholestasis and intrahepatic cholestasis of pregnancy 1 (ICP1) are taken as continuum of FIC1 deficiency and the protein

function is only partially impaired in them mostly related to missense mutations. In approximately 10% patients with PFIC1, only one mutated allele or no mutation is seen. In these patients, possible disease mechanisms include either the presence of mutations in regulatory sequences of the gene, or in the other genes involved in the transcription or protein trafficking of FIC1 protein [1, 6-8, 21, 22].

Clinical profile: A typical child with FIC1 disease presents with jaundice within the first few months of life. This is followed by diarrhea and growth failure. Pruritus is the dominant feature and is usually out of proportion to jaundice, it usually develops after 6 months of age after the neural pathways for concerted scratching are well developed. Biochemically, patients have conjugated hyperbilirubinemia, normal serum gamma-glutamyltranspeptidase (GGT), high serum bile acids, and mildly elevated transaminases. In view of wider 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, growth impairment beyond that attributable to cholestasis and delayed puberty and sexual development [1, 4, 6, 8, 13].

Histology: Histology reveals bland intracanalicular cholestasis without signs of significant hepatocyte injury. With disease progression, inflammation, fibrosis, bile duct proliferation, and cirrhosis develop. Transmission electron microscopy may demonstrate coarsely granular bile in the canaliculus. Liver biopsy usually shows normal histology or hepatocellular cholestasis and cholate injury, mostly centrilobular. Immunostaining for FIC1 has not been established for routine clinical use. However, there are surrogate markers of FIC1 deficiency like reduced canalicular staining for GGT, CD10, and carcinoembryonic antigen [1, 23].

Benign recurrent intrahepatic cholestasis (BRIC): Recurrent attacks of cholestasis, termed as BRIC, present as attacks of jaundice and pruritus separated by symptom-free intervals. These episodes may start at any age (1-50 years, usually before 20 years) and are associated with fatigue, malaise, anorexia, steatorrhea, dark-colored urine, and weight loss. There is no progression to cirrhosis or long-term complications of chronic liver disease. Attacks usually are preceded by a minor illness and consist of a preicteric phase of 2–4 weeks (characterized by malaise, anorexia, and pruritus) and an icteric phase that may last from 1 to 18 months. In some patients, hormonal factors such as the use of oral contraceptives or pregnancy, or antibiotics may be associated with precipitation of an attack. During the icteric phase, the concentrations of serum bile acid, bilirubin, and alkaline phosphatase are increased with low GGT, however in the intervening periods biochemistry is completely normal [1, 3].

Disease course and outcome: Early in the course of the disease when pruritus remains the chief and devastating symptom affecting the quality of life, partial external (PEBD) or internal biliary diversion (PIBD) is helpful. With advancement of liver disease, eventually these children may require liver transplantation [4, 6, 8].

BSEP DEFICIENCY (PFIC2)

Severe BSEP deficiency is the commonest form of PFIC worldwide [5-8, 24]. With defective or deficient BSEP, there is accumulation of bile acids within the hepatocyte with accompanying toxic damage and progression of disease.

Genotype-phenotype correlation: Although earlier reports did not suggest genotype-phenotype correlation, but this has been proved recently [8]. Severe phenotypes are often associated with mutations leading to premature protein truncation or failure of protein production. Milder phenotypes with reduced transport capacity of BSEP may be caused by mutations in one or both copies of this gene. True pathogenic mutation may be present on only 1 allele, although polymorphisms (such as p.V444A) may influence the levels of protein expression or function on the other allele and this means that such individuals have reduced function on both alleles. Thus, there may be reduced or absent function and defective translocation. It has been suggested that around 25% of ideal BSEP function is the threshold for patients at risk of cholestasis, but this may be influenced by drugs, pregnancy, viruses, malignancy, or other less recognized precipitants [1, 6, 25]. Variants of ABCB11 have also been associated with BRIC-2 drug-induced cholestasis and some patients of ICP, and these variants are milder, missense type and located in less conserved regions of the gene [1].

Two mutations, relatively common (58%) among European patients with BSEP deficiency (p.E297G or p.D482G), lead to some residual function in upto 45%. Patients with at least 1 copy of either of these mutations can present with complete PFIC-2 phenotype or a less severe phenotype. They also have been shown to have better outcomes, and improved responses to some treatments, compared with other patients with early-onset BSEP deficiency [6]. From the European cohort of BSEP, it was found that portal hypertension was more frequent and survival with native liver poor in those without D482G mutation in contrast to those with that mutation. Hence, D482G represents a more insidious and milder form of BSEP [26].

Genetic classification of BSEP: The results of multicentre NAtural course and Prognosis of PFIC and Effect of biliary Diversion (NAPPED) consortium comprising a cohort of 264 children with homozygous or compound heterozygous pathological ABCB11 mutations categorized BSEP patients on the basis of type of mutations (i) *BSEP1:* those with at least one copy of p.D482G or p.E297G (mildest phenotype with least severe disease), (ii) *BSEP2:* those with at least one missense mutation (yet not p.D482G or p.E297G), and (iii) *BSEP3:* those with mutations causing completely non-functional protein or total absence of BSEP expression on immunostaining. It was found that BSEP1 patients had better long-term outcomes with their native livers than BSEP2 and BSEP3 (20.4 versus 7.0 versus 3.5 years, respectively). This classification also has implication to decide management strategy for these patients as biliary diversion surgery was beneficial for BSEP1 and 2 patients, but not for patients with BSEP3 [27].

Clinical profile: Children with significant reduction in BSEP function generally present in the first few months of life, and manifest as neonatal or infantile cholestasis, growth failure, fat-soluble vitamin deficiencies, pruritus, high serum bile acids and moderate to severe elevation of transaminases and normal GGT. Spectrum, however, varies from mild intermittent cholestasis (BRIC2), isolated pruritus to rapidly progressive liver disease necessitating LT by first few years of life [4-8].

Histology: Liver histology shows marked intracellular cholestasis, usually obvious giant cell transformation [23]. A proportion of patients also show destructive bile duct damage leading to duct loss [24]. BSEP antibody staining is abnormal or absent in more than 90% of severe cases [1] (Picture 1).

Disease course and outcome: Several drugs like ursodeoxycholic acid (UDCA) and various other molecular chaperones have been tried for BSEP deficiency. However, the disease typically progresses to end-stage liver disease by first few years of life [4]. Diversion surgery is helpful before the development of advance fibrosis (Metavir stage <F3). 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. Good response to PEBD is seen in upto 76% of individuals with at least one copy of either of the variants p.E297G or p.D482G [28]. Many patients eventually require LT for debilitating pruritus affecting their quality of life, and before the development of end-stage liver disease [4, 6, 8, 28]. Despite early transplant, upto 15% of these children develop hepatocellular carcinoma, either clinically or at explant, and mostly under 5 years of age [29]. Due to selective expression of BSEP only in liver, unlike FIC1 protein, transplantation is the definite cure for the disease [1].

MDR3 DEFICIENCY (PFIC3)

MDR3 deficiency or PFIC3, encoded by ABCB4 gene is a type of cholangiopathy – biliary injury caused by elevated biliary bile acids [30]. Estimated incidence rate is 1:50,000 to 1:1,00,000. The protein transports phospholipid (chiefly phosphatidylcholine) from the inner to the outer leaflet of the canalicular membrane (floppase), which is then available for incorporation into bile micelles. Due to the deficiency of phospholipids in the bile, there are non-micellar free bile acids. These free bile acids have detergent action and cause injury to the cholangiocyte membranes ("toxic bile concept"). Hence, there is no retention of bile acids in the hepatocytes. Moreover, as biliary cholesterol solubilization also depends on appropriate concentration of bile acids and phospholipids, this mismatch also contributes to formation of extra- and intra-hepatic crystals and gallstones [31].

Genotype-phenotype correlation: Various mutations in the ABCB4 gene have been recognized and characterized as non-sense mutations and frameshift deletions leading to complete absence of protein (I),

missense leading to defective maturation of protein (II), activity (III), stability (IV) and variants without detectable effects. Heterozygotes for complete loss of function alleles have 50% function of the protein, which is sufficient to cause damage in some individuals [32]. Such individuals may be found to have some evidence of liver disease on evaluation in the first few decades but may remain asymptomatic, and later on present with end-stage liver disease in adulthood, or with hepatobiliary malignancy. Thus, heterozygous relatives of patients with MDR3 deficiency are therefore at increased risk of slowly progressive disease and should not be considered as suitable donors without proper screening [1].

Clinical profile: In the typical form of PFIC3 presentation, the disease presents as childhood cholestasis (median age 4.7 years), some patients present in infancy as conjugated hyperbilirubinemia. Symptoms include pruritus, hepatosplenomegaly, jaundice and features of portal hypertension. Rapid progression to liver cirrhosis and decompensation happens at an age between 3 to 15 years – half require LT around the end of first decade [4]. Even complete deficiency of MDR3 can take several years before presenting clinically, and hence some patients may present late in adolescence or adult life with mild to moderate jaundice, gall stones or hepatolithiasis. Transaminases are moderately elevated, but alkaline phosphatase and GGT are markedly high [32-34]. ABCB4 mutations also predispose adult patients to gall-bladder carcinoma and cholangiocarcinoma [32].

Histology: Liver biopsy shows cholangiolytic changes – bile ductular proliferation, hepatocellular and canalicular cholestasis, portal expansion and fibrosis [23]. In an infant, these histological features may simulate biliary atresia [34]. Immunohistochemical staining for MDR3 may be deficient (missense mutations) or absent (truncated mutations) [32] (Picture 1).

***Intrahepatic cholestasis of pregnancy (ICP)*:** ICP represents a milder spectrum of MDR3. This is the commonest liver disease during pregnancy and presents typically as pruritus starting at third trimester predominantly affecting hands and feet, with remission of cholestatic features within 2 weeks of delivery. Biochemical abnormalities include elevated fasting bile acid levels, transaminases, alkaline phosphatase and GGT. Conjugated hyperbilirubinemia is uncommon. This is associated with higher risk of premature delivery, meconium staining of amniotic fluid, respiratory distress and intrauterine death. Higher serum bile acids are related to increased fetal risk [35]. In a large European cohort of 563 pregnant ladies with ICP, single nucleotide polymorphisms in the genes ABCB4 (rs2109505) and ABCB11 (rs3815676) [36]. UDCA is indicated to alleviate pruritus, and early induction of labour is indicated by around 37 weeks. These ladies have increased risk of gallstones later in their lives [35].

Low phospholipid associated cholelithiasis (LCAP): LCAP is characterized by an increased risk of early development of gallstones in the gallbladder as well as within the liver (hepatolithiasis). Diagnosis is based on presence of two of the following: (i) biliary symptoms before the age of 40 years, (ii) detection of intrahepatic microlithiasis/sludge by ultrasound (hyperechoic foci), and (iii) recurrence of cholelithiasis after cholecystectomy. Diagnosis is confirmed by microscopic examination of endoscopically sampled hepatic or duodenal bile, which contains aggregated cholesterol crystals or microliths and reduced contents of phospholipids (in relation to bile acids). Sequencing of all exons of ABCB4 may reveal functionally relevant variants. In case of symptomatic gallstones, cholecystectomy with/without bile duct exploration or endoscopic retrograde cholangiography has to be performed. Hepatolithiasis may need localized liver resections for control of recurrent cholangitis [32].

Two less common forms of MDR3 deficiency are drug induced cholestasis and contraceptive induced cholestasis (CIC). Drugs especially some antibiotics and psychotropic drugs which inhibit P-glycoproteins can induce cholestatic form of liver injury in presence of ABCB4 mutations. Similarly, oral contraceptive pills can precipitate cholestasis particularly in those with personal or family history of ICP [32, 33].

Disease course and outcome: As there is mismatch in the bile salt and phospholipid pool in MDR3, PEBD is not a suitable option and has not been reported in these children. However, usage of UDCA early in the course of the disease or in milder forms has been shown to halt the progression of disease by its detergent action on the cholangiocyte membrane [34]. But the action is

limited due to the inability of UDCA to suppress the synthesis of endogenous bile salts, via FXR [15]. Liver transplantation is the definite treatment for severe MDR3 deficiency presenting as end-stage liver disease [1, 34].

Natural history and outcomes of FIC1, BSEP and MDR3 deficiencies:

The natural history and outcomes of these 3 commonest forms of PFIC have been studied in a recent review with 17 publications describing natural history or epidemiology and 5 publications describing their health-related quality of life (HRQoL). Pruritus was experienced by 11-100% of patients at presentation and by 76-100% of patients at follow-up. Pruritus is often debilitating, associated with abrasions, cutaneous mutilation, hemorrhage, and scarring and is grade >/=3+ on Whitington scale. Pruritus was identified as most bothersome symptom in PFIC – more often in types 1 and 2 (76-100%) versus type 3 (25-69%). These children have poor HRQoL as assessed by Pediatric Quality of Life Inventory (PedsQL) Measurement Model and Infant Dermatitis Scale. The HRQoL scores, physical health and psychosocial summary scores were poorer in comparison to their healthy peers [4].

PFIC1 children often presented with poor growth (~100%), diarrhea (61%), pancreatitis (8%), elevated sweat chloride (15%). In PFIC-2, there were deficiencies of vitamin-D in 3-22% and K in 8% (as bleeds) and cholelithiasis in 28%. There was rapid progression of histological fibrosis in children with PFIC2 in comparison to PFIC1. Among the patients undergoing LT, liver failure and/or HCC was detectable in about 60% of those with PFIC2 but in none of those with PFIC1. Untreated PFIC1 and 2 have mortality rates ranging from 0 to 87% and LT rates 40 to 100%. Reasons for mortality in untreated PFIC are infections, liver failure, bleeding (cerebral, gastrointestinal, splenic) and HCC. The common indications for liver transplantation in children with PFIC are failure of decompensated cirrhosis (78-97%), PEBD (29-67%), severe cholestasis and mutilating pruritus (7-42%), liver failure (32%), growth failure and development of HCC (10-26%) [4].

TJP2 MUTATIONS

The tight junction protein-2 (TJP2) or Zona occludens-2 are not part of the tight junction between the hepatocytes but is located in the cytoplasm and serve as a link between transmembrane tight junctions and actin cytoskeleton. TJP2 are closely associated with the tight junction proteins called Claudins [16]. Deficiency of Claudin-1 has been described, associated with a cholangiopathy termed neonatal icthyosis sclerosing cholangitis syndrome [37]. TJP2 is also known as Zona occludens 2(ZO2). Deficiency of TJP2 is associated with cholestasis, but not with cholangiopathy, suggesting that the tight junction barrier function is not badly disrupted. The mechanism of cholestasis is not very clear. As the tight junctions form a selective barrier and form a fence between the basolateral and canalicular membranes. These 2 membranes differ markedly in both protein and lipid composition, so disruption of TJP2 cause cholestasis by injury to membrane. Moreover, TJP2 has also been shown to travel to the nucleus, where it is transcriptionally active and inhibits cell cycle progression [16]. Homozygosity for a missense change manifests as hypercholanemia among the Amish population, with reduced penetrance. These patients did not manifest chronic liver disease [38]. On the other hand, biallelic mutations in TJP2 causing complete loss of TJP2 function cause severe progressive liver disease. These patients have very severe liver disease starting from early infancy with cholestasis, elevated bilirubin and transaminases and normal GGT. Most of these children require LT within first few years of life. From the description of 12 patients from King's College London, 11 with consanguinity, the median age of presentation was 2 months, and 9 required LT a median age of 4 (1.5-10) years. Due to the extrahepatic distribution of TJP2, respiratory and neurologic symptoms are often seen. Histology shows nonspecific features with intracellular cholestasis and giant cells. Immunohistochemical staining for TJP2 has been useful in identifying patients [9]. Patients with TJP2 deficiency and hepatocellular carcinoma have been described [39].

NR1H4 (FXR) MUTATIONS

FXR is a bile-acid activated nuclear receptor encoded by NR1H4 (nuclear factor subfamily 1 group H member 4) gene. As explained earlier in the text the central role of FXR in regulating biliary transport, it is easy to understand that FXR mutations with complete loss of its function cause severe cholestasis and liver damage. Four children were reported with homozygous mutations in NR1H4 gene who presented with

neonatal cholestasis, liver failure (coagulopathy), low-to-normal GGT, high transaminases, high alpha-fetoprotein levels and rapid progression to end-stage liver disease, One neonate presented with hydrops (ascites and pleural effusion) with intraventricular haemorrhage at birth. Two infants received LT at the age of 4.4 and 22 months, while other 2 died at 5 weeks and 8 months, respectively. Liver histology showed intralobular cholestasis with ductular reaction, hepatocellular ballooning, giant cell transformation and micronodular cirrhosis. There was absence of FXR and BSEP on immunostaining, the latter is attributed to the fact that FXR is required for BSEP expression on the canalicular membrane. Post-LT, one child had mild elevation of transaminases with histological steatosis. This was explained by lack of induction of FGF19 by intestinal FXR which remained deficient after LT [11].

MYOSIN 5B (MYO5B) MUTATIONS

Myosin 5B protein plays role in plasma membrane recycling, transcytosis, and epithelial cell polarization in multiple tissues, chiefly enterocytes, respiratory epithelial cells and hepatocytes. In liver, MYO5B interacts with RAB11A to facilitate normal trafficking of ABC transporter proteins, including BSEP, to the canalicular membrane [1]. Autosomal recessive mutations in MYO5B were initially identified in a proportion of patients with microvillus inclusion disease (MVID), a severe form of intractable diarrhea of infancy [10]. A subset of patients with MVID with MYO5B mutations developed cholestasis as well [40]. Recently, mutations in MYO5B have been reported in patients with isolated cholestasis, in the absence of obvious features of MVID [41-43]. The children with MYO5B related liver disease without MVID present with early childhood cholestasis, pruritus, hepatomegaly, failure to thrive, mild to moderate elevation of transaminases, elevated bile acid levels and low-normal GGT. Mutations were homozygous and compound heterozygous. Some children show response to UDCA and have transient or recurrent cholestatic features. Around half of the children require some form of biliary diversion (nasobiliary drainage or surgical diversion) [40-43]. A proportion may have resolved MVID [43]. Histology shows hepatocellular and canalicular cholestasis, giant cells, variable portal-periportal fibrosis and absence of ductular proliferation [41]. In one study with 28 MVID children, 8 developed cholestatic liver disease – 5 before and 3 after intestinal transplantation, the cholestasis improved only after biliary diversion procedures or after removal of the intestinal graft. Increase absorption of circulating bile acids after intestinal transplant was the possible reason for aggravation of liver disease [10]. The link between MVID and MYO5B related cholestasis is complex and has been addressed in a recent review. Of the total 133 reported patients of MVID, cholestatic liver disease was present in 37% and of MYO5B related MVID, the prevalence was 54%. MYO5B. Contrarily, only 21% of patients with liver disease had diarrhea. The postulated reason was that MYO5B mutations in isolated liver disease may not cause sufficient loss of MYO5B function to result in intestinal failure. Thus, varied presentations is due to unequal effects of MYO5B mutations in liver and intestine [40].

HEPATOCELLULAR CARCINOMA IN PFIC

Among the list of PFICs, children with BSEP deficiency are especially predisposed at a young age to develop HCC [29]. This happens due to persistent chronic inflammation leading to oncogenesis [44]. Studies from US and Europe showed that HCC occurs in 5-15% of children with BSEP deficiency at a young age (13 to 28 months) [6, 8, 26, 29]. Children with D482G mutations have less severe disease and portal hypertension, while HCC is common in those with non-D482G mutations [6]. From the cohort of 128 European children, single-strand conformation polymorphism analysis and sequencing of ABCB11 gene identified high risk of HCC (38% versus 10%) in children with presence of 2 protein-truncating mutations [26]. From the recent NAPPED cohort with classification of BSEP into 3 categories, the prevalence of HCC in BSEP1, 2 and 3 were found to be 4%, 7% and 34% [27]. Exome sequencing of the genomes of humans affected by BSEP and of *Mdr2*knock-out mice revealed that a very few somatic mutations accumulated over time in the cancer genes. This stands in contrast to adults with HCC as well as other malignancies where a number of mutations accumulate over a period of time. Further, in BSEP individuals and animals, there is massive gene amplification that affected components of signal transduction pathways, such as the ErbB, the PI3K/Akt and the mitogen-activated protein kinase

(MAPK) signalling pathways and in particular, activators of c-Jun-N terminal kinases (JNK) [45]. Another study which provided further pathophysiologic insights into BSEP mediated HCC showed that BSEP expression is severely diminished in HCC patients associated with alteration of farsenoid-X receptor (regulatory nuclear receptors) with increase in (FXR-α1/FXR-α2) ratio, the latter is induced by inflammation and may be reversible [46]. HCC has also been described in children with TJP2 deficiency again due to loss of hepatobiliary integrity and exposure of hepatocytes to detergent bile acids [39]. In MDR3 deficiency, HCC is rare but has been reported [47].

DIAGNOSIS AND DIFFERENTIALS

With the advancement of genetic testing, there is now limited role of histology and electron microscopy. Immunostaining is still used to quantitate the severity of deficiency of BSEP and MDR3 proteins. With the development of genetic technology, most of these diagnoses are nowadays genetic based and have guided the clinicians for management and prognosis also. 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, and is simpler than WGS. For cholestatic liver diseases, a targeted panel of genes can be sequenced which include all PFIC related genes, genes causing Alagille's syndrome (JAGGED1 and NOTCH2), Arthrogryposis, Renal Dysfunction and Cholestasis Syndrome, inborn errors bile acid synthesis, neonatal sclerosing cholangitis (Claudin1 and DCDC2) and Niemann-Pick type C disease. WES with a targeted approach is essential before subjecting these children for LT [1].

Low GGT versus high GGT cholestasis: The mechanism for the low levels of GGT in serum of patients of most of the PFICs except PFIC3 is not very clear. The GGT enzyme is normally bound to the canalicular membrane by a glycosyl phosphatidylinositol (GPI) anchor. In obstructive cholestasis as in biliary atresia, when excessive amounts of bile salts accumulate in the canalicular lumen under increased pressure, GGT is released from the membrane by detergent action and refluxes back into serum, possibly via leaky intercellular junctions. However, in all PFICs except MDR3 deficiency, alterations in lipid bilayer characteristics may lead to release of canalicular enzymes into bile. Immunohistochemical studies indicate that some canalicular proteins, including GGT and carcinoembryonic antigen, are poorly expressed at the canaliculus in PFIC1. On the other hand, in MDR3 there is cholangiocyte injury due to toxic bile acids leading to elevation of GGT [13]. Table 2 presents the differentials of low and high GGT cholestasis. In younger infants with low GGT, other differentials are bile acid synthetic defects, Aagenaes and arthrogryposis renal dysfunction and cholestasis (ARC) syndromes and metabolic enzyme defects. High GGT in very young infants require biliary atresia to be excluded. Other causes of high GGT are Alagille's syndrome, sclerosing cholangitis (neonatal, primary or secondary), congenital biliary stricture, inspissated bile duct syndrome and autoimmune-overlap syndrome [2, 3]. The differentials should be looked up in proper context and background.

MANAGEMENT

Management of all the forms of PFICs is focussed on control of pruritus, nutritional rehabilitation and surveillance and management of decompensation, portal hypertension and HCC (Figure 4) [13].

Control of pruritus: As discussed earlier that pruritus has multiple pathways, so numerous agents have been tried with a focus on promoting bile flow, decrease synthesis, binding, removal or replacement of toxic bile acids, altering metabolism of pruritogens and modifying itch perception at the level of central or peripheral nervous system [17].

Role of UDCA: UDCA is normally present in only small quantities (<3%) in human bile and is formed by 7β-epimerization of the primary bile salt, chenodeoxycholic acid, through the action of colonic bacteria – b-position confers hydrophilic nature to UDCA. The compound has multiple beneficial effects when used in patients with cholestasis – (i) replacement of toxic hydrophobic bile acids with hydrophilic UDCA, (ii) displacement of toxic bile acids from both the bile acid pool and hepatocellular membranes, and thus direct stabilization of the hepatocyte membrane (iii) direct hepatoprotective effect on hepatocytes, (iv) improvement of

mitochondrial oxidative phosphorylation and prevention of mitochondrial membrane permeability transition, (v) being poor at micelle formation and solubilization, and poorly absorbed from the proximal intestine, a large amount of orally administered UDCA reaches the terminal ileum where it interferes with the absorption of endogenous, hydrophobic and toxic bile acids – with oral administration UDCA concentration increases from 2 to 40%. (vi) direct hypercholeretic effect because of protonation of UDCA in the biliary ductule, and the protonated UDCA being lipophilic is rapidly absorbed by biliary epithelial cells prior to reaching the small intestine and is transported back to the liver (cholehepatic shunt), (vii) UDCA also increases bile salt-independent flow through a direct effect on cholangiocyte calcium-activated chloride secretion, resulting in bicarbonate-rich choloresis, and lastly (viii) immunomodulatory role by reducing immunologic injury associated with some cholestatic liver diseases – reduced expression of abnormal HLA-1 class proteins on hepatocytes [13]. UDCA is therapeutic for early and milder forms of MDR3 disease (response in pruritus and improvement in liver biochemistry in upto 79%), however the response rates are poor in most of the low-GGT cholestasis (<40-50%) [4].

Other treatments: A step-wise management of pruritus is mentioned in Figure 4 [13]. Phenobarbitone is also a choleretic which acts by increasing the bile acid independent fraction of bile flow, enhancing bile acid synthesis, inducing hepatic microsomal enzymes, and increasing hepatic Na+-K+-ATPase activity. Bile acid binding resins like cholestyramine or colestipol or colesevelam can be used to bind bile acids in the intestine, bloc enterohepatic circulation of bile acids, and thus decrease the pool. They also promote conversion of cholesterol into bile acids and thus stimulate choleresis. These drugs are given in juice or water either immediately before or after meals, when the bile secretion is maximal. However, the use is limited as other drugs should be avoided 2 hours before or after resins, and the tendency to worsen fat-soluble vitamin deficiencies [13]. Rifampicin is a Pregnane-X receptor pathway and induces Uridyl diphosphate glucoronosyl transferase 1A, CYP7A1, CYP3A4, MDR1, MRP2 and OSTβ, and thus helps in allaying pruritus in upto two-third of children with PFIC, although the response in partial in more than half of them [13, 15]. Various other agents act by modifying itch perception at the central level (opioid antagonists like naltrexone, nalmefene and naltrexone) or at the peripheral level (sertraline and ondansetron) [13]. Despite medical management, 60-100% of patients with PFIC1 and 2 have persistent pruritus and require diversion surgery [4].

Surgical diversion: Refractory pruritus not responding to medical management often requires surgery in the form of biliary diversion. Table 3 presents outcomes of diversion surgeries in children with PFIC1 and 2. Diversion surgeries are indicated for low-GGT cholestasis and Alagille's syndrome and not for MDR3 deficiency [48-51]. Patients should be considered for diversion only in the absence of advanced fibrosis (Metavir <F3, Ishak <F4) [50]. The basis for all these procedures is to interrupt enterohepatic circulation of bile salts, and thus allaying pruritus and improve liver biochemistry. Various surgical procedures used are mentioned below:

1. Partial external biliary diversion (PEBD): This is the most often used diversion procedure. In this procedure, bile is diverted from the gallbladder to the jejunal conduit (10-15 cm in length) connecting the gallbladder to the abdominal wall via a permanent cutaneous stoma, thus interrupting the enterohepatic circulation of bile acids. Bile collected in the stoma bag (120-200 ml/day) is discarded [13]. There are reports of creation of PEBD laparoscopically [52]. PEBD has been shown to improve growth, liver biochemistry, reverse and prevent progression of fibrosis and thus reduce disease progression in upto 80% of children with PFIC1 and 2 [48-50]. However, the procedure may fail in 25-71% [4]. From the largest multicentric cohort of children, median ages at PEBD for FIC1 and BSEP were 1.6 and 2.3 years with sustained improvement in pruritus in 57% and 44%. In the BSEP group, the response was better in those with D482G and E297G mutations [28]. Specifically, in the BSEP cohort of patients, surgical diversion is associated with

increased survival in those with BSEP1 or 2 (hazard ratio 0.50) than in those with BSEP3. Further, a low serum bile acid concentration <102 μmol/L or decrease of at least 75% shortly after diversion surgery predicted survival with native liver >/=15 years post-diversion [27].

2. Partial internal biliary diversion (PIBD): As PEBD cause persistent biliary fistula and is cosmetically not a good surgery, various types of internal biliary diversion surgeries have been devised which are more acceptable to the patients and their families. Some of these procedures are cholecystojejunocolonic, cholecystoileocolonic or cholecystoappendicocolonic anastomosis or cholecystocolostomy. In the cholecystojejunocolonic anastomosis, 15-20 cm jejunal conduit is anastomosed proximally in a terminolateral fashion to gall bladder and distally to the colon. PIBD can also be performed laparoscopically. Although cosmetically favourable, PIBD carries risk of complications like intestinal obstruction, ascending cholangitis and osmotic diarrhea due to increased bile acid load to colon [13, 51].
3. Ileal bypass or exclusion: In this procedure, there is construction of side to side ileocolic anastomosis leading to diversion of bile acids directly into colon. This is an alternative rescue option to PEBD and should be offered cautiously, only to patients who cannot benefit from PEBD [13, 28].
4. Total biliary diversion (TBD): In PEBD, the common bile duct remains intact, so a small fraction of the bile is still excreted into the duodenum, which is reabsorbed in the terminal ileum, contributing to persisting cholestasis and pruritus. TBD has been done in children with refractory pruritus and has been shown to completely abolish or significantly reduce pruritus. The study proposed TBD as a surgical technique for non-cirrhotic patients with low-GGT cholestasis with failed PEBD or PIBD [53]. Moreover, TBD has been advocated for children with FIC1 disease who develop intestinal symptoms after LT and is sometimes done at the time of LT [54].

Nutritional rehabilitation: Most of these children have poor growth due to persistent cholestasis, increased catabolic state, anorexia, splenomegaly due to portal hypertension and presence of ascites. These children need supplementation with fat-soluble vitamins 3-5 times of recommended dietary allowance (RDA), water soluble vitamins 2-3 times of RDA, calories 125% of RDA based on weight for height at 50th centile and proteins 2-3 g/kg/day. Medium chain triglycerides should comprise 60-70% of the calories provided by fats in the diet – these are better absorbed in children with cholestasis, reduce steatorrhea, improve energy balance and promote growth. Essential major and trace elements are needed in children with suspected deficiencies: calcium (25-10 mg/kg/day upto 800-1200 mg/day), phosphorus (25-50 mg/kg/day upto 500 mg/day), magnesium (1-2 meq/kg/day), zinc (1 mg/kg/day), selenium (1-2 μg/kg/day) and iron (5-6 mg/kg/day). Night-time drip feeds as nasogastric feeds are required in children with poor weight gain. Some patients may need an insertion of percutaneous endoscopic gastrostomy tube [13]. These children need careful surveillance every 2 weeks for growth to decide need for nutritional intervention.

Liver transplantation: Various indications for LT in PFIC are decompensated end-stage liver disease, refractory pruritus, unsuccessful biliary diversion and severe growth failure [4, 6, 8, 28]. From the multicentric European and American cohort of patients with PFIC1 and 2 with 102 children (60 FIC1 and 42 BSEP deficiency), 57 children required LT. It was shown that there was longer survival with native liver without developing cirrhosis in children with FIC1 deficiency and those with BSEP D482G or E297G mutations in comparison to those with other BSEP mutations. Transplantation improves cholestasis in all group of patients. Overall outcomes were good with 5% mortality and 9% retransplantation. Five BSEP and 4 FIC1 patients received living donor LT – 7 from obligate heterozygous parents, however the outcome was not different. Graft steatosis and diarrhea were more common in FIC1 thatn BSEP patients (90.5% and 81% versus 6.4% and 7%). Also, there was mild

elevation of transaminases and platelets in FIC1 patients after LT. FIC1 patients remained at lower end of their growth centiles 1 year post-LT (35% and 31% above 3rd centile for weight and height) in contrast to BSEP patients (88% and 90% above 3rd centile for weight and height), and had a trend towards delayed puberty [28].

Post-transplant diarrhea and graft steatosis in FIC1 disease: There is high prevalence of diarrhea and graft steatosis (73%) progressing to steatohepatitis (64%) within a year of transplantation in FIC1 patients. The possible explanation for exacerbation of diarrhea post-LT is because ATP8B1 gene product dysfunction is decompensated on the intestinal side after continuous restoration of bile flow and bile acid secretion leading to high bile acid load in the intestine causing refractory diarrhea and subsequently graft steatosis. This is also explainable by the fact that the diarrhea and steatosis improves with usage of bile acid absorptive resin. Another explanation for diarrhea is exocrine pancreatitis insufficiency [55]. Total biliary diversion surgery after or at the time of LT helps in alleviating diarrhea in these children and is used by some centres [54].

Post-transplant recurrence of disease in BSEP deficiency: Some children develop recurrence of BSEP disease after LT. This happens more often in children with splice-site and premature stop codon mutations with complete absence of BSEP before LT leading to insufficient auto-tolerance against BSEP after LT. These allo-reactive antibodies are directed specifically against one extracellular loop of the BSEP protein, and which block the function of the normal protein in the transplanted liver. Due to humoral nature of this phenomenon, the derangements in liver functions in these children are sometimes refractoriness to changes in immunosuppressive medications [56, 57]. There are reports on successful usage of B-cell depletion therapies i.e. combination of rituximab (monoclonal anti-CD20 antibody), intravenous immunoglobulin and plasmapheresis, followed by resolution of recurrence [58].

Surveillance:

Surveillance for decompensation and portal hypertension: Children with BSEP and TJP2 mutations need close monitoring for presence of decompensation. Regular outpatient visits every 4-6 weeks are required for early detection of decompensation [6, 8, 9]. FIC1 and MYO5B children may need less frequent monitoring [6, 8, 40]. Screening for varices should be performed in all children with persistent splenomegaly and/or platelet counts <100,000/mm^3. MDR3 children, who usually present late, need careful follow-up for decompensation as well as portal hypertension. Repeat endoscopy with absent, small or large varices should be performed at 6, 6, 3 monthly intervals.

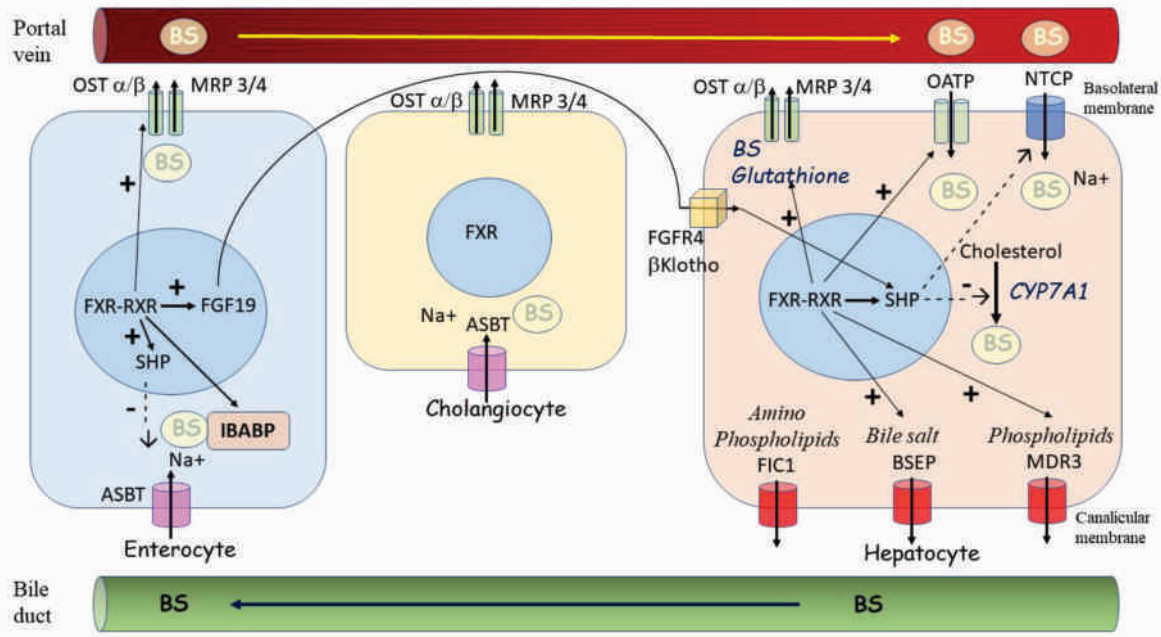
Surveillance for HCC: BSEP deficiency children, especially with BSEP3 (completely non-functional protein or total absence of BSEP on immunostaining) and those with TJP2 mutations require 3-monthly surveillance for HCC with ultrasound and serum alfa-fetoprotein levels. Children with cirrhosis with other types of PFIC need 6-monthly surveillance for HCC [44].

NEW TREATMENT TARGETS

Table 4 shows various newer treatment approaches for children with PFIC based on modulating action of bile acids (PPAR-α, TGR5 agonists), reducing intestinal uptake of bile acids (ASBT inhibitors), removal of pruritogens (ultraviolet-B, plasmapheresis, molecular adsorption reabsorption system, MARS), reducing synthesis of bile acids (FXR agonists) or altering metabolism of pruritogens (PXR agonists, Ultraviolet-B) [13, 15, 59-64].

Conclusion

PFIC are autosomal recessive heterogenous group of cholestatic disorders characterized by pruritus, growth failure, hepatosplenomegaly and poor quality of life. With advancement in genetics and basic science research, these disorders are now well identified and characterized. Genetic based classification helps in guiding management and prognosis of these children. Surgical diversion techniques serve as a definite or bridging treatment for these children. Liver transplantation offers complete cure for these entities but with risk of diarrhea in FIC1 and recurrence of disease in BSEP. Future research in this field is ongoing to identify newer genetic entities causing cholestasis and therapeutic agents directed against the hepatotoxic effects of bile acids.



Legends for Figures 1-3 and Picture 1:

Figure 1: Biliary transport and role of FXR. Diagram showing biliary transporters in the hepatocytes, cholangiocytes and enterocytes and the central role of Farnesoid-X receptor in regulating bile acid synthesis and transport via FGF19 and SHP proteins [12, 14, 15]. (Abbreviations: ASBT = Apical sodium bile acid transporter, BS = Bile salt, BSEP = Bile salt export pump, CYP = Cytochrome P enzyme, FIC1 = Familial intrahepatic cholestasis type 1, FXR = Farnesoid X receptor, FGF19 = Fibroblast growth factor 19, FGFR4 = Fibroblast growth factor receptor 4, IBABP = Intestinal bile acid binding protein, MDR = Multidrug resistance protein, MRP = Multidrug resistance associated protein, Na+ = Sodium ion, NTCP = sodium taurocholate cotransporting polypeptide, OATP = organic anion transporting polypeptide, OST = organic solute transporters, RXR = Retinoid X receptor).

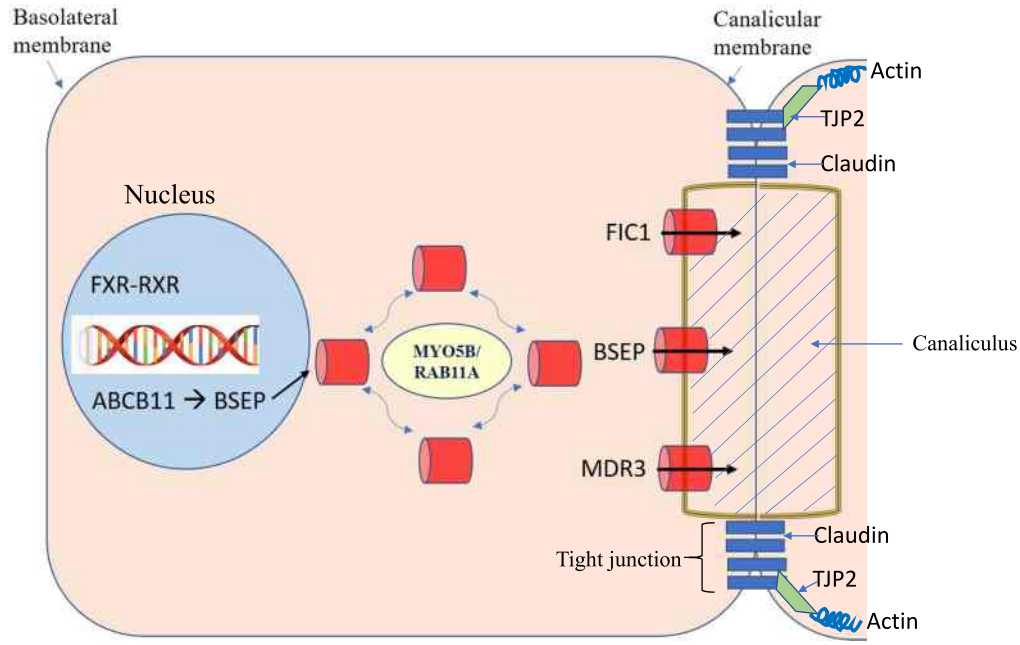


Figure 2: Biliary transporters in PFIC. Diagram showing various biliary transporters involved in PFICs. Farnesoid X receptor mediates the expression of transporters like BSEP which then are trafficked via Myosin-5B and RAB11A recycling endosome pathway to the plasma membrane [9, 10, 12, 15]. (Abbreviations: BSEP = Bile salt export pump, FIC1= Familial intrahepatic cholestasis type 1 protein, FXR = Farnesoid X receptor, MDR3 = Multidrug resistance protein-3, MYO5B = Myosin 5B protein, RXR = Retinoid X receptor, TJP2 = Tight junction protein 2).

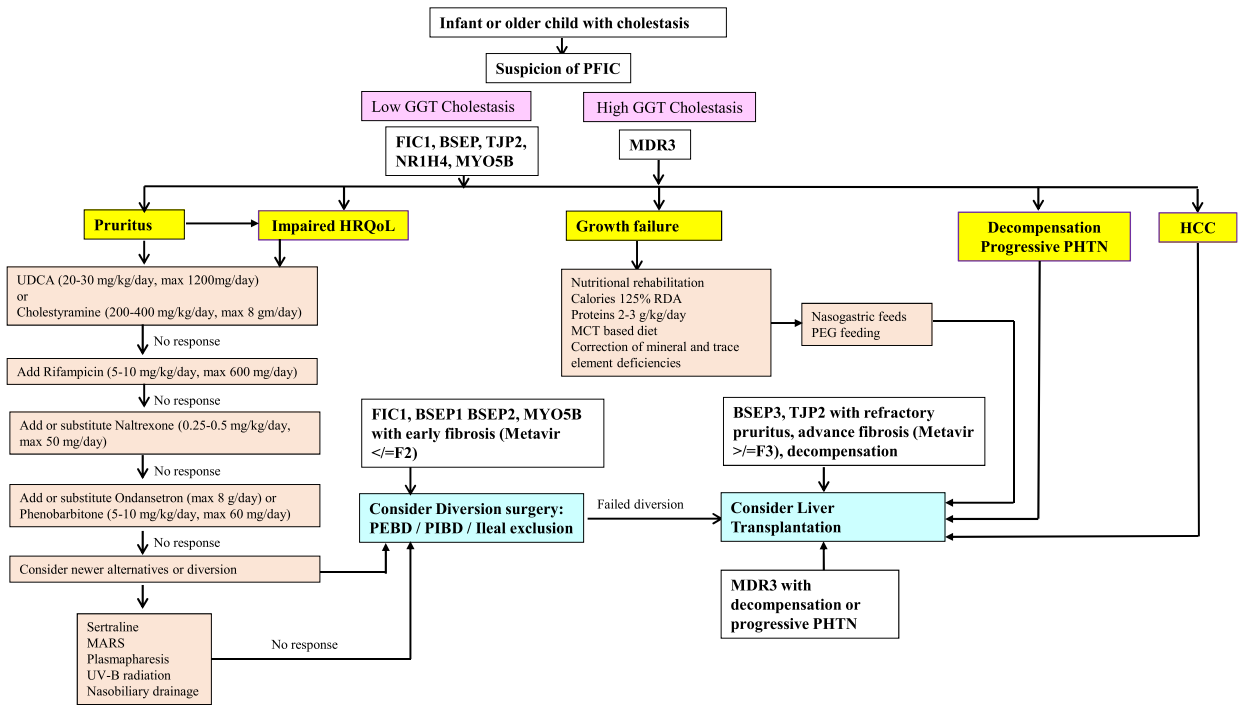
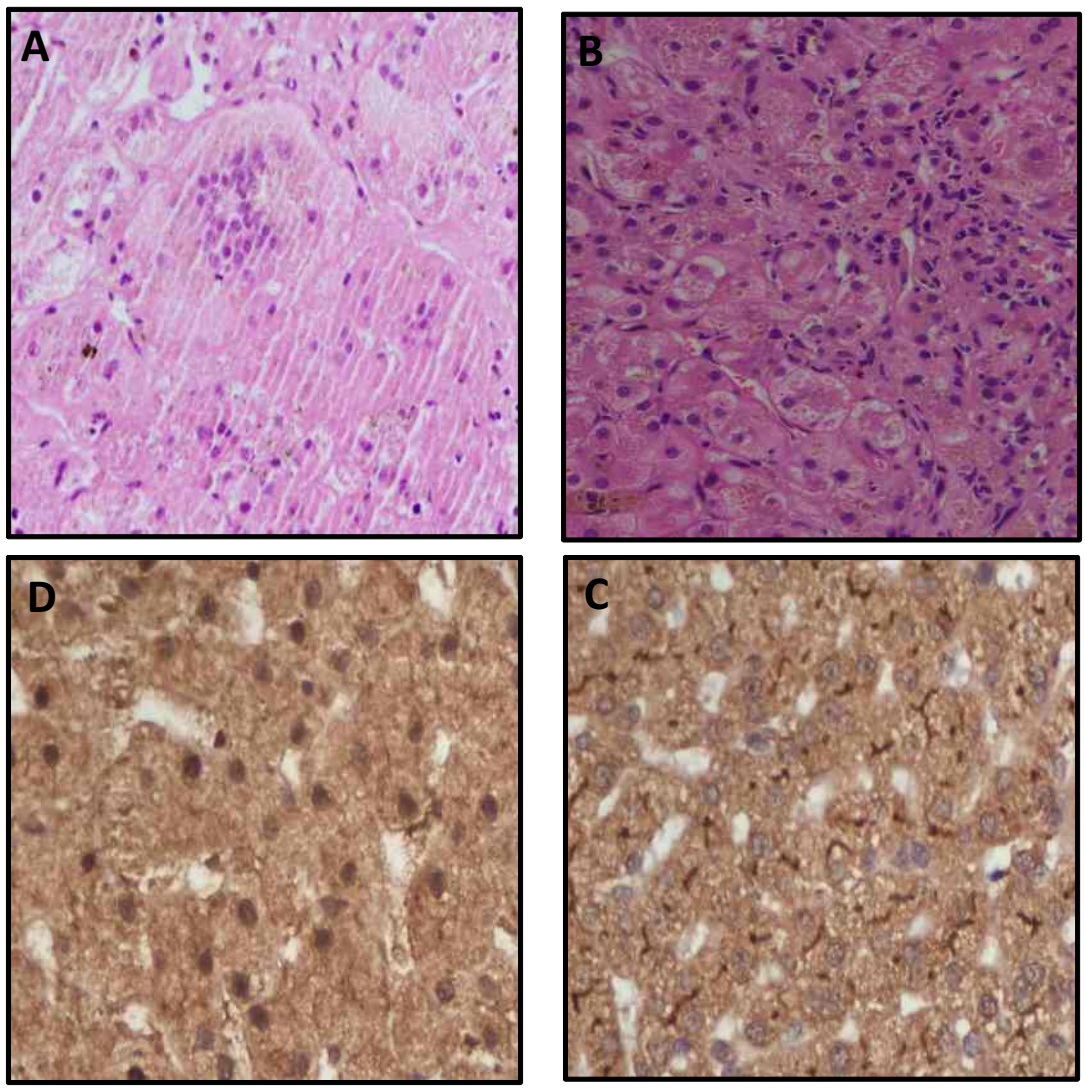


Figure 3: Management algorithm for children with PFIC. Refractory pruritus not responding to medications should be considered for biliary diversion surgery. Indications for liver transplant are failed diversion, presence of decompensation or progressive portal hypertension. Patients with BSEP3 (non-D482G, non-E297G), TJP2 and MDR3, those with advanced fibrosis or HCC should be considered for transplantation [1-9, 11, 27, 28, 34, 40]. [Abbreviations: BSEP = Bile salt export pump deficiency, BSEP1 = those with at least one copy of p.D482G or p.E297G (mildest phenotype with least severe disease), BSEP2 = those with at least one missense mutation (yet not p.D482G or p.E297G), BSEP3: those with mutations causing completely non-functional protein or total absence of BSEP expression on immunostaining, FIC1 = Familial intrahepatic cholestasis type 1 deficiency, HCC = Hepatocellular carcinoma, HRQoL = Health related quality of life, MARS = Molecular adsorbent recirculating system, MCT = Medium chain triglycerides, MDR3 = Multidrug resistance protein-3 deficiency, MYO5B = Myosin 5B protein mutations, PEG = Percutaneous endoscopic, PHTN = Portal hypertension, RDA = Recommended dietary allowance, TJP2 = Tight junction protein 2 mutations, UDCA=Ursodeoxycholic acid, UV-B=Ultraviolet B]



.**Picture 1: Histological features in children with PFIC.** Panel A shows liver biopsy of a 4-month-old boy with PFIC2 with evidence of giant cell hepatitis in (400X, Haematoxylin and Eosin, H&E stain). Panel B shows liver biopsy of a 9-month-old boy with PFIC2 with evidence of hepatocellular and canalicular cholestasis, feathery degeneration and periportal inflammation (400X, H&E stain). Panel D shows liver biopsy of a 10 year old boy with PFIC3 with features of cholestasis, growth failure, pruritus, and portal hypertension showing absent MDR3 on immunostaining in comparison to the control liver biopsy of a child with hepatitis-B (Panel C) (400X, MDR3 Immunostain).

References:

1. Bull LN, Thompson RJ. Progressive Familial Intrahepatic Cholestasis. Clin Liver Dis. 2018;22 (4):657–69.
2. Jacquemin E. Progressive familial intrahepatic cholestasis. Clin Res Hepatol Gastroenterol. 2012; 36(SUPPL.1):S26–35.
3. Srivastava A. Progressive familial intrahepatic cholestasis. J Clin Exp Hepatol. 2014;4(1): 25–36.
4. Baker A, Kerkar N, Todorova L, Kamath BM, Houwen RHJ. Systematic review of progressive familial intrahepatic cholestasis. Clin Res Hepatol Gastroenterol. 2019;43(1): 20–36.
5. Agarwal S, Lal BB, Rawat D, Rastogi A, Bharathy KG, Alam S. Progressive Familial Intrahepatic Cholestasis (PFIC) in Indian Children: Clinical Spectrum and Outcome. *J Clin Exp Hepatol*. 2016;6(3):203-208.
6. Pawlikowska L, Strautnieks S, Jankowska I, Czubkowski P, Emerick K, Antoniou A, et al.

Differences in presentation and progression between severe FIC1 and BSEP deficiencies. J Hepatol. 2010;53(1):170–8.

7. Sharma A, Poddar U, Agnihotry S, Phadke SR, Yachha SK, Aggarwal R. Spectrum of genomic variations in Indian patients with progressive familial intrahepatic cholestasis. BMC Gastroenterol. 2018;18(1):1–10.
8. Davit-Spraul A, Fabre M, Branchereau S, Baussan C, Gonzales E, Stieger B, et al. ATP8B1 and ABCB11 Analysis in 62 children with normal gamma-glutamyl transferase Progressive Familial Intrahepatic Cholestasis (PFIC): Phenotypic differences between PFIC1 and PFIC2 and natural history. Hepatology. 2010;51(5):1645–55.
9. Sambrotta M, Strautnieks S, Papouli E, Rushton P, Clark BE, Parry DA, et al. Mutations in TJP2 cause progressive cholestatic liver disease. Nat Genet. 2014;46(4):326–8.
10. Girard M, Lacaille F, Verkarre V, Mategot R, Feldmann G, Grodet A, et al. MYO5B and bile salt export pump contribute to cholestatic liver disorder in microvillous inclusion disease. Hepatology. 2014;60(1):301–10.
11. Gomez-Ospina N, Potter CJ, Xiao R, Manickam K, Kim MS, Kim KH, et al. Mutations in the nuclear bile acid receptor FXR cause progressive familial intrahepatic cholestasis. Nat Commun. 2016;7:1–8.
12. Kullak-Ublick GA, Stieger B, Meier PJ. Enterohepatic Bile Salt Transporters in Normal Physiology and Liver Disease. Gastroenterology. 2004;126 (1 SUPPL. 1) : 322-42.
13. Gupta NA, Karpen SJ, Feranchak AP, Suchy FJ, Sokol RJ, Sundaram SS, et al. Mechanisms of bile formation and cholestasis. Medical and Nutritional management of cholestasis in infants and children. Familial hepatocellular cholestasis. In: Suchy FJ, Sokol RJ, Balistreri WF, editors. Liver Disease in Children. 4th edition. New York: Cambridge University Press; 2014.p24-31, 111-139, 199-215.
14. Sticova E, Jirsa M, Pawłowska J. New Insights in Genetic Cholestasis: From Molecular Mechanisms to Clinical Implications. Can J Gastroenterol Hepatol. 2018;2018.
15. Beuers U, Trauner M, Jansen P, Poupon R. New paradigms in the treatment of hepatic cholestasis: from UDCA to FXR, PXR and beyond. *J Hepatol*. 2015;62 (1 Suppl) : S25-S37.
16. Sambrotta M, Thompson RJ. Mutations in TJP2, encoding zona occludens 2, and liver disease. Tissue Barriers. 2015;3(3):1–5.
17. Beuers U, Kremer AE, Bolier R, Elferink RP. Pruritus in cholestasis: facts and fiction. *Hepatology*. 2014;60(1):399-407.
18. Eppens EF, Van Mil SWC, De Vree JML, Mok KS, Juijn JA, Oude Elferink RPJ, et al. FIC1, the protein affected in two forms of hereditary cholestasis, is localized in the cholangiocyte and the canalicular membrane of the hepatocyte. J Hepatol. 2001;35(4):436–43.
19. Cai SY, Gautam S, Nguyen T, Soroka CJ, Rahner C, Boyer JL. ATP8B1 Deficiency Disrupts the Bile Canalicular Membrane Bilayer Structure in Hepatocytes, But FXR Expression and Activity Are Maintained. Gastroenterology. 2009;136(3):1060-1069.e4.
20. Chen F, Ananthanarayanan M, Emre S, Neimark E, Bull LN, Knisely AS, et al. Progressive Familial Intrahepatic Cholestasis, Type 1, Is Associated with Decreased Farnesoid X Receptor Activity. Gastroenterology. 2004;126(3):756–64.
21. Klomp LWJ, Vargas JC, Van Mil SWC, Pawlikowska L, Strautnieks SS, Van Eijk MJT, et al. Characterization of mutations in ATP8B1 associated with hereditary cholestasis. Hepatology. 2004;40(1):27–38.
22. Giovannoni I, Callea F, Bellacchio E, Torre G, De Ville De Goyet J, Francalanci P. Genetics and molecular modeling of new mutations of familial intrahepatic cholestasis in a single Italian center. PLoS One. 2015;10(12):1–13.
23. Morotti RA, Suchy FJ, Magid MS. Progressive familial intrahepatic cholestasis (PFIC) Type 1, 2, and 3: A review of the liver pathology findings. Semin Liver Dis. 2011;31(1):3–10.
24. Meena BL, Khanna R, Bihari C, Rastogi A, Rawat D, Alam S. Bile duct paucity in childhood-spectrum, profile, and outcome. *Eur J Pediatr*. 2018;177(8):1261-1269.
25. Soroka CJ, Boyer JL. Biosynthesis and trafficking of the bile salt export pump, BSEP: Therapeutic implications of BSEP mutations. Mol Aspects Med. 2014;37:3–14.
26. Strautnieks SS, Byrne JA, Pawlikowska L,

Cebecauerová D, Rayner A, Dutton L, et al. Severe bile salt export pump deficiency: 82 different ABCB11 mutations in 109 families. Gastroenterology. 2008 Apr;134(4):1203-14.

27. Van Wessel DBE, Thompson RJ, Gonzales E, et al. Genotype correlates with the natural history of severe bile salt export pump deficiency. *J Hepatol*. 2020;73(1):84-93.
28. Bull LN, Pawlikowska L, Strautnieks S, Jankowska I, Czubkowski P, Dodge JL, et al. Outcomes of surgical management of familial intrahepatic cholestasis 1 and bile salt export protein deficiencies. Hepatol Commun. 2018;2(5):515–28.
29. Knisely AS, Strautnieks SS, Meier Y, Stieger B, Byrne JA, Portmann BC, et al. Hepatocellular carcinoma in ten children under five years of age with bile salt export pump deficiency. Hepatology. 2006 Aug;44(2):478-86.
30. De Vree JML, Jacquemin E, Sturm E, Cresteil D, Bosma PJ, Aten J, et al. Mutations in the MDR3 gene cause progressive familial intrahepatic cholestasis. Proc Natl Acad Sci U SA. 1998;95(1):282–7.
31. Park HJ, Kim TH, Kim SW, Noh SH, Cho KJ, Choi C, et al. Functional characterization of ABCB4 mutations found in progressive familial intrahepatic cholestasis type 3. Sci Rep. 2016;6(February):1–9.
32. Reichert MC, Lammert F. ABCB4 Gene Aberrations in Human Liver Disease: An Evolving Spectrum. Semin Liver Dis. 2018;38(4):299–307.
33. Sundaram SS, Sokol RJ. The multiple facets of ABCB4 (MDR3) deficiency. Curr Treat Options Gastroenterol. 2007;10(6):495–503.
34. Schatz SB, Jüngst C, Keitel-Anselmo V, Kubitz R, Becker C, Gerner P, et al. Phenotypic spectrum and diagnostic pitfalls of ABCB4 deficiency depending on age of onset. Hepatol Commun. 2018;2(5):504–14.
35. Webb GJ, Elsharkawy AM, Hirschfield GM. Editorial: The etiology of intrahepatic cholestasis of pregnancy: Towards solving a monkey puzzle. Am J Gastroenterol. 2014;109(1):85–8.
36. Dixon PH, Wadsworth CA, Chambers J, et al. A comprehensive analysis of common genetic variation around six candidate loci for intrahepatic cholestasis of pregnancy. *Am J Gastroenterol*. 2014;109(1):76-84.
37. Grosse B, Cassio D, Yousef N, et al. Claudin-1 involved in neonatal ichthyosis sclerosing cholangitis syndrome regulates hepatic paracellular permeability. Hepatology 2012;55(4):1249–59.
38. Carlton VE, Harris BZ, Puffenberger EG, et al. Complex inheritance of familial hypercholanemia with associated mutations in TJP2 and BAAT. *Nat Genet*. 2003;34(1):91-96.
39. Zhou S, Hertel PM, Finegold MJ, Wang L, Kerkar N, Wang J, et al. Hepatocellular carcinoma associated with tight-junction protein 2 deficiency. Hepatology. 2015 Dec;62(6):1914-6.
40. van IJzendoorn SCD, Li Q, Qiu Y, Wang J, Overeem AW. Unequal effects of MYO5B mutations in liver and intestine determine the clinical presentation of low-GGT cholestasis . Hepatology. 2020;0–2.
41. Gonzales E, Taylor SA, Davit-Spraul A, Thébaut A, Thomassin N, Guettier C, et al. MYO5B mutations cause cholestasis with normal serum gamma-glutamyl transferase activity in children without microvillous inclusion disease. Hepatology. 2017;65(1): 164–73.
42. Qiu YL, Gong JY, Feng JY, Wang RX, Han J, Liu T, et al. Defects in myosin VB are associated with a spectrum of previously undiagnosed low γ-glutamyltransferase cholestasis. Hepatology. 2017;65(5):1655–69.
43. Cockar I, Foskett P, Strautnieks S, Clinch Y, Fustok J, Rahman O et al. Mutations in Myosin 5B (MYO5B) in Children with Early Onset Cholestasis. J Pediatr Gastroenterol Nutr. 2020. Published ahead of Print.
44. Khanna R, Verma SK. Pediatric hepatocellular carcinoma. *World J Gastroenterol*. 2018;24 (35):3980-3999.
45. Iannelli F, Collino A, Sinha S, Radaelli E, Nicoli P, D'Antiga L, et al. Massive gene amplification drives paediatric hepatocellular carcinoma caused by bile salt export pump deficiency. Nat Commun. 2014 May 13;5:3850.
46. Chen Y, Song X, Valanejad L, Vasilenko A, More V, Qiu X, et al. Bile salt export pump is dysregulated with altered farnesoid X receptor isoform expression in patients with

hepatocellular carcinoma. Hepatology. 2013 Apr;57(4):1530-41.

47. Vij M, Shanmugam NP, Reddy MS, Govil S, Rela M. Hepatocarcinogenesis in multidrug-resistant P-glycoprotein 3 deficiency. Pediatr Transplant. 2017 May;21(3).

48. Whitington PF, Freese DK, Alonso EM, Schwarzenberg SJ, Sharp HL. Clinical and biochemical findings in progressive familial intrahepatic cholestasis. *J Pediatr Gastroenterol Nutr*. 1994;18(2):134-141.

49. Englert C, Grabhorn E, Richter A, Rogiers X, Burdelski M, Ganschow R. Liver transplantation in children with progressive familial intrahepatic cholestasis. *Transplantation*. 2007;84(10):1361-1363.

50. Yang H, Porte RJ, Verkade HJ, De Langen ZJ, Hulscher JB. Partial external biliary diversion in children with progressive familial intrahepatic cholestasis and Alagille disease. *J Pediatr Gastroenterol Nutr*. 2009;49(2):216-221.

51. Erginel B, Soysal FG, Durmaz O, Celik A, Salman T. Long-term outcomes of six patients after partial internal biliary diversion for progressive familial intrahepatic cholestasis. *J Pediatr Surg*. 2018;53(3):468-471.

52. Metzelder ML, Bottländer M, Melter M, Petersen C, Ure BM. Laparoscopic partial external biliary diversion procedure in progressive familial intrahepatic cholestasis: A new approach. Surg Endosc Other Interv Tech. 2005;19(12):1641–3.

53. Van Der Woerd WL, Kokke FT, Van Der Zee DC, Houwen RH. Total biliary diversion as a treatment option for patients with progressive familial intrahepatic cholestasis and Alagille syndrome. J Pediatr Surg. 2015;50(11): 1846–9.

54. Alrabadi LS, Morotti RA, Valentino PL, et al. Biliary drainage as treatment for allograft steatosis following liver transplantation for PFIC-1 disease: A single-center experience. Pediatric Transplantation. 2018 Jun;22(4): e13184. DOI: 10.1111/petr.13184.

55. Miyagawa-Hayashino A, Egawa H, Yorifuji T, et al. Allograft steatohepatitis in progressive familial intrahepatic cholestasis type 1 after living donor liver transplantation. Liver Transpl 2009;15(6):610–8.

56. Maggiore G, Gonzales E, Sciveres M, Redon MJ, Grosse B, Stieger B, et al. Relapsing features of bile salt export pump deficiency after liver transplantation in two patients with progressive familial intrahepatic cholestasis type 2. J Hepatol. 2010;53(5):981–6.

57. Keitel V, Burdelski M, Vojnisek Z, Schmitt L, Häussinger D, Kubitz R. De novo bile salt transporter antibodies as a possible cause of recurrent graft failure after liver transplantation: a novel mechanism of cholestasis. *Hepatology*. 2009;50(2):510-517.

58. Lin HC, Alvarez L, Laroche G, Aldana HM, Pfeifer K, Schwarz K et al. Rituximab as Therapy for the Recurrence of Bile Salt Export Pump Deficiency After Liver Transplantation. Liver transplantation 2013;19:1403–1410.

59. Nevens F, Andreone P, Mazzella G, et al. A Placebo-Controlled Trial of Obeticholic Acid in Primary Biliary Cholangitis. *N Engl J Med*. 2016;375(7):631-643.

60. Corpechot C, Chazouillères O, Rousseau A, et al. A Placebo-Controlled Trial of Bezafibrate in Primary Biliary Cholangitis. *N Engl J Med*. 2018;378(23):2171-2181.

61. Harrison SA, Rossi SJ, Paredes AH, et al. NGM282 Improves Liver Fibrosis and Histology in 12 Weeks in Patients With Nonalcoholic Steatohepatitis. *Hepatology*. 2020;71(4):1198-1212.

62. Shneider BL, Spino C, Kamath BM, et al. Placebo-Controlled Randomized Trial of an Intestinal Bile Salt Transport Inhibitor for Pruritus in Alagille Syndrome. *Hepatol Commun*. 2018;2(10):1184-1198.

63. Pares A, Herrera M, Aviles J, Sanz M, Mas A. Treatment of resistant pruritus of cholestasis with albumin dialysis. Combined analysis of patients from three centers. *J Hepatol.* 2010; **53**: 307-31.

64. Decock S, Roelandts R, Steenbergen WV, et al. Cholestasis-induced pruritus treated with ultraviolet B phototherapy: an observational case series study. *J Hepatol*. 2012;57(3):637-641.

Table 1: Different types of PFICs – Genetic defects, transporters and clinical course (Based on references 1-9, 11, 28, 33-35, 40)

Mutated protein (Disease)	FIC1 deficiency (PFIC1)	BSEP deficiency (PFIC2) (	MDR3 deficiency (PFIC3)	TJP2 mutations	NR1H4 (FXR) mutations	MYO5B mutations
Gene	ATP8B1	ABCB11	ABCB4	TJP2	NR1H4	MYO5B
Gene location	18q21-22	2q24	7q21	9q21.11	12q23.1	18q21.1
Function	Translocation of aminophospholipids from outer to inner leaflet of lipid bilayer (flippase)	Bile salt export	Translocation of phosphatidylcholine from inner to outer leaflet of lipid bilayer (floppase)	Intracellular anchoring leading to sealing of canaliculi	Expression of biliary transporters like BSEP and MDR3	Intracellular trafficking and localization of apical membrane transporters
GGT	Normal	Normal	High	Normal	Normal	Normal
Elevation of Transaminases	Mild	Moderate to severe	Mild to moderate	Moderate	Moderate	Mild
Gallstones	-	++	+++	-	-	-
Recurrence cholestasis	+ (BRIC1)	+ (BRIC2)	++	-	+	-
Liver failure	-	++	-	++	Early (as infantile liver failure)	-
Extrahepatic features	++ (Diarrhea, pancreatitis, deafness, respiratory distress)	-	-	+ (Respiratory, Neurological)	-	Diarrhea (as part of MVID)
HCC	-	+++	+	++	-	-
Histology	Bland canalicular cholestasis, Coarse granular canalicular bile on EM	Giant cell transformation, duct loss	Bile ductular proliferation, cholangiolytic changes	Bland cholestasis	Intralobular cholestasis, ductular reaction, giant cell transformation	Giant cell transformation, Hepatocellular and canalicular cholestasis
Biliary bile acids	Low	Very low	Normal	-	-	-
Biliary phospholipids	Normal	Normal	Low	-	-	-
Progression to ESLD	Slow	Rapid	Progression variable	Rapid	Very rapid	Slow
Natural history	Growth failure, severe pruritus	Growth failure, pruritus, HCC, ESLD requiring LT in early childhood	PHTN, growth failure, cholestasis, gallstones, ESLD by end of first or second decade	Similar but rapid course than PFIC2, Increase HCC risk	Requires LT in infancy	Associated MVID may need multi-visceral transplant
Unique features	Post-transplant diarrhea and graft steatosis	Risk of recurrence post-LT due to allo-antibody against BSEP	Association with ICP, Drug induced cholestasis, CIC, LPAC	-	Post-LT steatosis	Isolated liver disease may be protective against MVID

Abbreviations: BRIC = Benign recurrent intrahepatic cholestasis, BSEP = Bile salt export pump, CIC = Contraceptive induced cholestasis, ESLD = End-stage liver disease, FIC1 = Familial intrahepatic cholestasis type 1, FXR = Farnesoid X receptor, GGT = Gamma-glutamyl transpeptidase, HCC = Hepatocellular carcinoma, ICP = Intrahepatic cholestasis of pregnancy, LPAC = Low phospholipid associated cholestasis, LT = Liver transplantation, MDR3 = Multidrug resistance protein-3, MVID = Microvillus inclusion disease, MYO5B = Myosin-5 B, NR1H4 = Nuclear receptor subfamily 1 group H member 4, PFIC = Progressive familial intrahepatic cholestasis, TJP2 = Tight junction protein 2.

Table 2: Differential diagnosis of PFIC based on GGT and age of presentation.

Low or Normal GGT **(FIC1 / BSEP / TJP2 / FXR / MYO5B)**	**High GGT** **(MDR3)**
Bile acid synthetic defects (infant)	Biliary atresia (infant)
Aagenaes syndrome (infant or older child)$	Alagille's syndrome (infant or older child)^
ARC syndrome (infant)#	Inspissated bile duct syndrome (infant)
Metabolic disorders – Galactosemia, Tyrosinemia, Hereditary fructose intolerance (infant)@	Neonatal sclerosing cholangitis (infant)
	Congenital biliary stricture (infant)
	Secondary sclerosing cholangitis* (toddler)
	Cystic fibrosis (older child)
	Primary sclerosing cholangitis (older child)
	Overlap syndrome (older child/adolescent)

$Aaganes syndrome: presents as lymphedema and cholestasis, ^Alagille's syndrome: characteristic features are triangular facies, bulbous nose, murmur of peripheral pulmonary stenosis, butterfly vertebra, posterior embryotoxon, ductal paucity on liver histology, #ARC syndrome: Arthrogryposis renal dysfunction and cholestasis syndrome, presents as cholestasis, diarrhea, renal tubular dysfunction, contractures, @Metabolic enzyme defects: usually infants are sick with coagulopathy, decompensation, diarrhea and vomitings, *secondary sclerosing cholangitis: usually develops in the setting of Langerhan's cell histiocytosis, HIV, Tuberculosis or cystic fibrosis.

Table 3: Studies on diversion surgeries and their outcomes in PFIC.

Author (Year) [Reference]	No of patients	Type of diversion	Key findings
Whitington PF et al (1994) [48]	33 PFIC	PEBD in 14 Partial Ileal bypass in 2	PEBD: Relief of cholestasis completely (64%), partially (7%), secondary LT (29%)
Englert C et al (2007) [49]	42 PFIC (26 type 2, 16 type 3)	17 PEBD	Successful PEBD in 29%, Referred for LT (76%)
Yang H et al (2009) [50]	11 PFIC 3 Alagille's	PEBD	Pruritus relieved completely in in 50%, partially in 25%, Bile salts and growth improved in most patients, Bile salts reduced in those with early fibrosis but not with advanced fibrosis
Erginel et al (2018) [51]	6 PFIC	PIBD	Decrease in serum bile acids, bilirubin and transaminases, Improvement in pruritus 5 (83%) symptom-free at 6 years follow-up, 1 had refractory pruritus, died after LT
Bull LN et al (2018) [28]	102 PFIC (60 FIC1, 42 BSEP)	57 PEBD 6 Ileal exclusion 57 LT	Sustained improvement in pruritus: No difference between FIC1 or BSEP, BSEP common D482G or E297G mutations showed 76% response & BSEP other mutations 33% (OR for sustained response = 8.1) Median time from PEBD to LT: BSEP common D482G or E297G mutations > FIC1 > BSEP other mutations Progression to cirrhosis: BSEP other mutations (33%) > BSEP common D482G or E297G (9.5%) mutations > FIC1 (0%) Need for LT: BSEP other mutations (70%) > FIC1 (27%) > BSEP common D482G or E297G (16%)
Van der Woerd WL et al (2015) [53]	4 PFIC 1 Alagille's	TBD	PFIC: marked improvement clinically and biochemically Alagille's: pruritus improved but cholestasis persisted

Abbreviations: BSEP = Bile salt export pump deficiency (PFIC2), FIC1 = Familial intrahepatic cholestasis type 1 (PFIC1), LT = Liver transplantation, PEBD = Partial external biliary diversion, PIBD = Partial internal biliary diversion, PFIC = Progressive familial intrahepatic cholestasis.

Table 4: Newer potential treatment targets for children with cholestatic liver diseases including PFICs [15].

Therapeutic agents	Target	Action	Remarks
Obeticholic acid [59]	FXR	Agonist	Used in adult patients with PBC, increase pruritus (POISE trial), Improved inflammatory markers, ALP and bilirubin, decreased C4 bile acids
All-trans retinoic acid	RXR	Agonist	Therapeutic benefits not yet proven
Bezafibrate [60] Fenofibrate Ciprofibrate	PPARα	Agonist	Used in adult patients with PBC (BEZURSO trial), Improved liver biochemistry, fatigue, pruritus and fibrosis; Insertion of MDR3 into canalicular membrane, anti-inflammatory effects
Rifampicin Statins Corticosteroids	PXR	Agonist	Induction of CYP7A1, UGT1A1, MDR1, MRP2, MRP3, OSTβ, Rifampicin improves itching
NGM282 (FGF19 analogue) [61]	FGFR4	Activator	Multiple roles in bile acid, carbohydrate and lipid metabolism, Used in NASH patients with improvement in liver fibrosis
Int777	TGR5	Agonist	Inhibits proinflammatory cytokine production, migration and phagocytic activity of macrophages and Kupffer cells, improves intestinal barrier function
Maralixibat [62]	ASBT	Inhibitor	Inhibits bile acid absorption, 1-point reduction in pruritus when used in Alagille's syndrome
Vitamin D	VDR	Agonist	Stimulation of bile acid detoxification enzymes (CYP3A4 and SULT2A1)
norUDCA	-	Choleretic	Cholehepatic shunting allows targeted anti-inflammatory, anti-fibrotic and anti-prolifeartive effects to injured ducts
MARS [63]	-	Removes pruritogens	Used in adults with median 2 sessions (1-5), Reduces pruritus and bile acids
Ultraviolet-B light phototherapy [64]	-	Chemically modify pruritogens	Works at a wavelength of 290-320 nm, Used in adults, 60% reduction in pruritus, Risk of skin cancer, keratitis, cataract, infertility

Abbreviations: ALP = Alkaline phosphatase, ASBT = Apical sodium bile acid transporter, CYP = Cytochrome P enzyme, FXR = Farnesoid X receptor, FGF19 = Fibroblast growth factor 19, FGFR4 = Fibroblast growth factor receptor 4, MARS = Molecular adsorbent recirculating system, MDR = Multidrug resistance protein, MRP = Multidrug resistance associated protein, NASH = Non-alcoholic fatty liver disease, OST = organic solute transporters, PPARa = Peroxisome proliferator-activated receptor alpha, PXR = Pregnane X receptor, RXR = Retinoid X receptor, SULT2A1 = Sulfotransferase Family 2A Member 1, TGFR5 = Transforming growth factor receptor 5, UDCA = Ursodeoxycholic acid, UGT = Uridylylglucoronosyl transferase, VDR = Vitamin-D receptor.