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Progressive Familial Intrahepatic Cholestasis

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Additional information is available at the end of the chapter

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1. Introduction

Neonatal cholestasis is one of the commonest presentations in the field of pediatric hepatology and gastroenterology and constitutes the major indication for liver transplantation below two years of age. Unfortunately, in spite of being common, fewer categories are amenable to curative or palliative therapy. Moreover, delayed referral to specialized centers is still a problem adding a more difficulty to neonatal cholestasis management. Hepatobiliary surgery is a major line of therapy in some etiologies of neonatal cholestasis. Biliary atresia, choledochal cyst, spontaneous perforation of the bile duct and inspissated bile syndrome are among the commonest known causes for hepatobiliary surgeons. However, there is less orientation about other causes, resulting in progression to cirrhosis and end stage liver disease without being diagnosed. One of these is the progressive familial intrahepatic cholestasis (PFIC) group of diseases [1].

PFIC is an autosomal recessive liver disorder characterized by an intrahepatic cholestasis due to bile canalicular transport defects. It is subdivided into three types with slightly different clinical, biochemical and histological features. PFIC types 1, 2 and 3 are due to mutations in *ATP8B1* (adenosine triphosphatase, type 8B, member 1), *ABCB11* (adenosine triphosphate-binding cassette, subfamily B, member 11) and *ABCB4* (adenosine triphosphate-binding cassette, subfamily B, member 4) genes, respectively. Each of these genes encodes a hepatocanalicular transporter which is essential for the proper secretion and formation of bile [2].

PFIC1 and PFIC2 usually appear in the first months of life, whereas onset of PFIC3 may also occur later in infancy, in childhood or even during young adulthood. The shared main clinical manifestations in all types are cholestasis and pruritus. PFIC represents 10-15 % of causes of cholestasis in children and 10-15% of indications of liver transplantations in children [3].

In this chapter, we want to highlight the etiology, pathophysiology, clinical presentation and the role of surgery in the management of this disease category, especially that medical therapy is of limited value in a magnitude of cases. Moreover, liver transplant is not without significant side effects. So, raising the orientation about this not uncommon condition will help in timely surgical intervention and improving patients' outcome.

2. Historical background

This disorder was first described by Clayton in 1965, and was termed Byler's disease after an American Amish kindred in which it was discovered [4]. Clinical features included severe pruritus, steatorrhea, poor growth and progression to cirrhosis in early childhood. A prominent finding was a low or normal serum gamma glutamyl transpeptidase (GGT), which was discordant with the severe cholestasis. Since its discovery, similar clinical features were described in non-Amish children. Therefore, the more descriptive term, PFIC, is preferred [5].

However, this PFIC nomenclature is not always entirely satisfactory. A preferable term is "bile canalicular transport disorders," especially as it has become apparent that these genetic disorders have numerous clinical phenotypes across all age brackets. For example, benign recurrent intrahepatic cholestasis (BRIC) and intrahepatic cholestasis of pregnancy (ICP) can occur in association with abnormalities in any of the three affected genes [2,6]. However, the PFIC nomenclature is still in use due to its popularity in the literature.

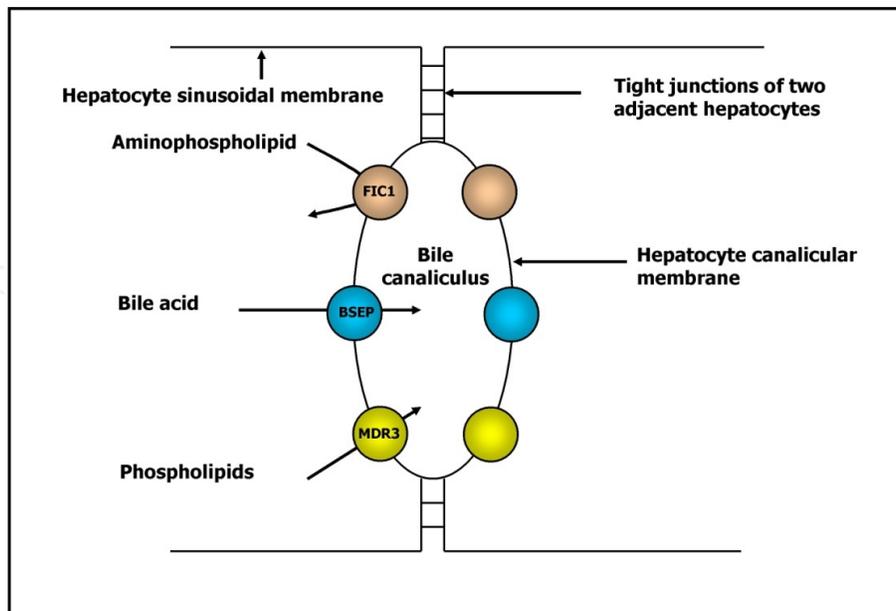
Benign recurrent intrahepatic cholestasis, first described in 1959, is an intermittent form of intrahepatic cholestasis characterized by variable periods of intense pruritus often associated with jaundice [7]. The age of onset is variable, but it typically occurs during childhood or adolescence. The severity and duration of attacks also vary and triggering features are not well known. The benign designation of BRIC refers to the general lack of progressive liver disease, although the pruritus is far from benign during an intense episode [8-10].

As the clinical spectrum between BRIC and PFIC (formerly named Byler's disease) may be a continuum, thus the historical nomenclature of Byler's disease and BRIC may be outdated [11]. So, many clinicians now refer to all these diseases in a general sense as ATP8B1, ABCB11 and ABCB4 deficiency diseases to express the wide continuum of disease severity between the PFIC and BRIC phenotypes [2].

3. Etiology and pathophysiology

3.1. PFIC 1 (ATP8B1 "FIC-1" deficiency)

PFIC1 is an autosomal recessive disease caused by mutations in *ATP8B1* (formerly named *FIC1*) gene on chromosome 18, locus q21-22. This gene encodes a transporter localized on the canalicular membrane of hepatocytes (Figure 1), named FIC1 (ATP8B1), a P-type ATPase [12].



Abbreviations: FIC1: familial intrahepatic cholestasis 1; BSEP: bile salt export pump; MDR3: multidrug resistance protein 3.

Figure 1. A schematic representation of the hepatocyte with its canalicular membrane transporters involved in bile formation. FIC1 is an aminophospholipid flippase, encoded by the *ATP8B1 (FIC1)* gene. BSEP (bile salt export pump) (formerly sister of P-glycoprotein "SPGP") is a bile acids transporter to the bile canaliculus lumen against a high concentration gradient. It is encoded by the *ABCB11 (BSEP)* gene. The MDR3 is a phospholipid transporter. It is encoded by the *ABCB4 (MDR3)* gene.

The most widely accepted hypothesis for FIC1 function is that of an aminophospholipid flippase, translocating phospholipids such as phosphatidylserine from the outer to the inner leaflet of the plasma membrane [13]. So, deficiency of FIC1 in the hepatocyte results in the loss of asymmetric distribution of phospholipids in the canalicular membrane, decreasing both membrane stability and function of transmembrane transporters including the bile salt export pump (BSEP) and, as such, causing bile salt retention in hepatocytes with consequent defective bile formation; resulting in cholestasis [14-16].

Different studies have shown that *ATP8B1* deficiency is associated with diminished FXR (farnesoid X receptor) activity. The FXR is a nuclear receptor that is highly expressed in the liver and regulates bile acid homeostasis so as to reduce its hepatocyte toxicity. Diminished FXR activity leads to upregulation of bile acid synthesis, reduced expression of the canalicular BSEP, and increased expression of the ileal apical sodium dependent bile acid transporter (ASBT). The net effect of these changes would be increased synthesis of bile acids and diminished its canalicular excretion, coupled with enhanced reabsorption of intestinal bile acids, yielding marked hepatocyte bile acid overload [17,18].

ATP8B1 is abundantly expressed in a wide variety of tissues such as the small intestine, bladder and stomach and to a lesser extent also in the liver and pancreas. This results in the multitude of the extrahepatic manifestations such as the hearing loss, pancreatitis and diarrhea, found in patients with *ATP8B1* deficiency [19,20]. Over 50 distinct mutations in

ATP8B1 are described. The mutations G308V found in Amish, D554N found in Inuits and I661T are amongst the most frequently detected [21,22].

In vitro studies showed that ATP8B1 deficiency due to common missense mutations such as G308V, D554N and I661T, can be regarded as a protein folding disease, with different degrees of retention of the mutant protein in the endoplasmic reticulum, resulting in a decreased protein expression at the plasma membrane [23]. The pathophysiologic concept of being a protein folding disease can be used in new therapeutic interventions [24]. Incubation at a reduced temperature could improve proper folding of some of the mutated proteins. Similarly, the pharmacological chaperone 4-phenylbutyrate acid (4-PBA) could stabilize misfolded proteins, partially restoring cell surface expression [25].

Mutations in ATP8B1 are also responsible for:

1. Greenland Eskimo cholestasis (Nielsen syndrome) [26].
2. Benign recurrent intrahepatic cholestasis-1 (BRIC1) [12].
3. Intrahepatic cholestasis of pregnancy-1 (ICP1) [27].
4. Down regulation of CFTR (cystic fibrosis transmembrane regulator): ATP8B1 is highly expressed in biliary epithelial cells, and when it is abnormal in PFIC1, CFTR down regulation in cholangiocytes has been reported which could contribute to impairment of bile secretion [28].

3.2. PFIC 2 (ABCB11 "BSEP" deficiency)

PFIC2 is an autosomal recessive disease caused by mutations in the *ABCB11* (formerly named *BSEP*) gene encoding the BSEP, a liver-specific adenosine triphosphate (ATP)-binding cassette transporter formerly known as sister of P-glycoprotein (SPGP). BSEP is located in the hepatocyte canalicular membrane (Figure 1). *ABCB11* gene is located on chromosome 2, locus q24 [29-31].

The defective canalicular BSEP expression leads to markedly diminished bile salt secretion. This leads to bile secretory failure with secondary retention of bile salts and other biliary constituents in the hepatocytes leading to progressive liver damage and progressive cholestasis. BSEP deficiency represents also a phenotypic continuum between BRIC2 and PFIC2. Different mutations may cause different kinds of BSEP dysfunction, including protein lack, misfolded protein, or protein not delivered from the Golgi to the bile canalicular membrane [31].

Generally missense mutations, e.g. E297G or D482G, lead to a less severe phenotype than mutations that are predicted to result in premature protein truncation or total failure of protein production [31,32]. In vitro, the residual transport function of mutant proteins correlates with the phenotypic differences between BRIC2 and PFIC2, with generally a diminished function in BRIC2 mutants, while complete abolishment is more often seen in PFIC2 mutants [33].

Heterozygous *ABCB11* mutations have also been identified in cases of ICP (ICP2) [34], drug induced cholestasis [35] and transient neonatal cholestasis [36].

3.3. PFIC 3 (ABCB4 "MDR3" deficiency)

PFIC3 is an autosomal recessive disorder due to mutations in the *ABCB4* (formerly named *MDR3*) gene located on chromosome 7, locus q21, which codes for the class III multidrug resistance P-glycoprotein (MDR3). MDR3 is located exclusively on the canalicular membrane of the hepatocyte and serves as a phospholipid translocator (Figure 1) essential for biliary phospholipid (e.g. phosphatidylcholine "PC") secretion [37].

PC in bile normally protects cholangiocytes from bile salt toxicity by forming mixed micelles with it. However, a mutation of the *ABCB4* gene results in decreased biliary PC secretion and high biliary bile salt -to-PC ratio, leading to bile duct injury (cholangitis and ductular proliferation). Also, a decreased biliary PC concentration leads to high biliary cholesterol -to-PC ratio. The high biliary cholesterol saturation promotes crystallization of cholesterol and the lithogenicity of bile [2,38].

Whereas biliary bile salt concentrations are normal in patients with PFIC3, serum bile salt levels are elevated. It is explained by:

1. Downregulation of the bile acid importers to the hepatocyte, NTCP (Na⁺/taurocholate cotransporting polypeptide) and OATP (organic acid transporting polypeptide) [39].
2. Upregulation of the bile acid exporter from hepatocyte at the sinusoidal membrane, MRP4 (multidrug resistance-related protein 4), mediating bile salt efflux into serum [40].

Over 45 disease-causing mutations in *ABCB4* have been identified [41]. Children with missense mutations seem to have a less severe phenotype, with later onset of disease, slower progression and better response to treatment, as compared to patients with mutations leading to a truncated protein [42]. Possibly this is due to residual transport activity in MDR3 protein affected by missense mutations.

Heterozygous mutations in the *ABCB4* gene can also cause or predispose for a variety of other liver diseases, such as adult biliary cirrhosis, cholelithiasis, transient neonatal cholestasis, drug induced cholestasis and ICP. Mutations can even lead to a cascade of several phenotypes in one patient, indicating the wide phenotypical spectrum of *ABCB4* deficiency [43,44].

A small proportion of PFIC phenotypes are not due to mutations in these three genes and therefore additional genes might be involved [2,45].

4. Clinical picture

Mutations in *ATP8B1* and *ABCB11* can result both in progressive cholestatic disease termed PFIC1 and PFIC2, as well as in episodic cholestasis, referred to as BRIC type 1 and 2 respectively. This suggests that PFIC and BRIC are the two ends of a clinical spectrum, with different degrees of severity in between. Therefore, these diseases are preferably referred to as *ATP8B1* deficiency and *ABCB11* deficiency. While mutations in *ABCB4* can result in progressive cholestatic disease only designated PFIC type 3. Similarly PFIC3 is best designated as *ABCB4* deficiency. Heterozygous mutations in any of these three genes can also be associated with ICP. It is a transient form of cholestasis, characterized by the onset of pruritus during pregnancy, with postnatal resolution [2].

Pruritus is the prominent clinical feature of PFIC; however, until an episode of jaundice intervenes, the diagnosis is often overlooked. Even then, because of the rarity of the condition, children sometimes receive a misdiagnosis of obstructive jaundice caused by the occasionally associated choledocholithiasis in PFIC types 2 and 3 [5].

4.1. PFIC1 "Byler's disease"

- Cholestasis is a major clinical sign in PFIC1 as in all PFIC forms. It usually appears in the first months of life in patients with PFIC1, and is characterized by recurrent episodes of jaundice, which become permanent later in the course of the disease [3]. The variable clinical features are:
 1. *Jaundice*: It presents with conjugated hyperbilirubinemia in the first 3–6 months of life. The degree of jaundice may vary [46].
 2. *Pruritus*: It is the dominant feature in the majority of patients and is often out of proportion to the level of jaundice [46]. It may initially vary in intensity and may be exacerbated during intercurrent illness. Pruritus may not be noticed until 6 months of age because the neural pathways necessary for concerted scratching are not fully developed. However, affected infants often are irritable and sleep poorly with onset of cholestasis. Scratching is usually evident first as digging at the ears and eyes, which are the first areas to show evidence of excoriation. By one year of age, patients may show generalized mutilation of skin, usually most severe on the extensor surfaces of the arms and legs and on the flanks of the back. The pruritus is very disabling and often responds poorly to medical therapies [12,45].
 3. *Hepatomegaly* is present early in life and persists with progression to cirrhosis. The rate of progression to cirrhosis is variable, but usually develops in early childhood without treatment. With progression to cirrhosis splenomegaly develops.
 4. *Fat-soluble vitamin deficiencies*, including rickets, may be severe.
 5. *Extrahepatic disorders* [19]:
 - Persistent diarrhea with fat malabsorption and protein loss, leading to poor growth and short stature.
 - Bouts of pancreatitis.
 - Recurrent pneumonia may also compromise growth.
 - Sensorineural hearing loss may occur.
- As it has been mentioned before, ATP8B1 deficiency can lead to a continuum of disease severity ranging from the progressive form PFIC1 to the recurrent form, BRIC1. BRIC1 will be discussed briefly in the next paragraphs.

BRIC is an intermittent form of intrahepatic cholestasis characterized by variable periods of intense pruritus often associated with jaundice, separated by symptom-free intervals. The benign designation of BRIC refers to the general lack of progressive liver disease, al-

though the pruritus is far from benign during an intense episode [10]. Two types of BRIC are present according to the gene defect. BRIC1 is due to a mutation of *ATP8B1* gene and BRIC2, due to *ABCB11* gene mutations.

The age of presentation of the first attack of jaundice ranges from 1–50 years, but jaundice usually occurs before the age of twenty years. 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 1–18 months. In some patients, hormonal factors such as the use of oral contraceptives and pregnancy have been associated with precipitation of an attack [10,47]. Patients may have severe coughing during episodes, as is seen sometimes in patients with PFIC1 [48].

During the icteric phase, the concentrations of serum bile acid, bilirubin, and alkaline phosphatase (ALP) are increased. Serum GGT concentration, however, remains low. Liver biopsy results are very benign, often showing no pathologic change even during an episode. Some specimens show hepatocellular cholestasis and cholate injury, mostly centrilobular. During the asymptomatic period, all parameters (clinical, laboratory and liver histology) are normal [49].

4.2. PFIC2 "Byler's syndrome"

- PFIC2 affected children differ from those with PFIC1 in some important respects:

The initial presentation and the evolution seem to be more severe than PFIC1, with permanent jaundice from the first months of life and rapid appearance of cirrhosis and liver failure within the first years of life [3].

They do not have extrahepatic involvement such as pancreatitis or diarrhea [45].

Early hepatocellular carcinoma (before one year of age) may complicate the course of PFIC2 [3]. Up to 15% of the patients with *ABCB11* deficiency will develop hepatocellular carcinoma (HCC) or cholangiocarcinoma. Close surveillance for hepatobiliary malignancy is therefore warranted in these patients [31,32,45].

At diagnosis, the cholestasis in *ABCB11* deficiency results in a more detectable fat-soluble vitamin deficiency manifestations [45].

The development of cholelithiasis in approximately one third of the patients, probably due to the low bile salt concentration in bile, secondary to impaired BSEP function, which might cause supersaturation of cholesterol [32].

- Patients fitting the phenotype of BRIC have been described with mutations in *ABCB11*. They are called BRIC2 and are characterized by:

The age of onset and total number of recurrent episodes were highly variable. Cholelithiasis occurred in many patients with BRIC2. Several patients had a relatively early onset of the disease and developed permanent cholestasis as adults after initial periods of recurrent attacks.

Occasionally BRIC will progress to the more severe and permanent form of PFIC, indicative of a clinical continuum, with intermediate phenotypes between mild and progressive disease [2,11].

4.3. PFIC3 "MDR3 deficiency"

Mutations in the *ABCB4* gene can cause or predispose to a variety of liver diseases with different age of presentation. Moreover, it can even lead to a cascade of several phenotypes in one patient, indicating the wide phenotypical spectrum of *ABCB4* deficiency [43].

1. *PFIC3*: it is characterized by:
 - *Cholestasis* developing within the first year of life in about one third of patients and rarely in the neonatal period. It may also manifest later in infancy, in childhood or even in young adulthood [3,45].
 - *Pruritus* occurs less frequently than in the other types of PFIC and is usually mild.
 - *Jaundice* may be less prominent than pruritus.
 - *Height and weight* may be below normal as the disease progresses.
 - *Hepatomegaly*, and at later stages splenomegaly, as a manifestation of portal hypertension is often observed. Liver disease tends to evolve slowly to biliary cirrhosis with or without overt cholestatic jaundice [42,50].
 - *Cholelithiasis* may develop in older children.
 - *No extrahepatic features* or occurrence of malignancies are described in association with PFIC3 [42,50].
2. *Adult biliary cirrhosis*: gastrointestinal bleeding due to portal hypertension and cirrhosis may be the presenting symptom in adolescent or young adult patients [3].
3. *ICP*: some cases of ICP have been associated with heterozygous mutations in *ABCB4* [43].
4. Heterozygous mutations in the *ABCB4* gene can also cause or predispose for transient neonatal cholestasis and drug induced cholestasis [44].

5. Diagnosis

Diagnosis is dependent firstly on suspicion. The most alarming point making PFIC in the scope of diagnosis is the presence of significant pruritus out of proportion to the level of jaundice especially in the setting of low GGT. However, accurate diagnosis is dependent on a constellation of a clinical, biochemical, radiological, histopathological, immunohistochemical studies and finally can be confirmed by genetic testing for mutations (Table 1).

1. *Biochemical parameters*:

- *Serum GGT* is repeatedly normal or low in PFIC1 & PFIC2, while it is elevated in PFIC3 often more than ten times the normal value. In PFIC1 and PFIC2, the serum GGT concentration may increase to greater than 100 IU/L in patients receiving microsomal inducers such as phenobarbital and rifampicin [51].

The mechanism for the low serum concentration of GGT in PFIC1 and 2 is not clear. GGT is normally bound to the canalicular membrane by a glycosyl phosphatidyl inositol (GPI) anchor. In obstructive cholestasis, 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 PFIC and BRIC types 1 and 2, the reduced concentrations of biliary bile acids preserve canalicular GGT localization. This explanation is not entirely satisfactory as serum GGT is elevated in most other forms of intrahepatic cholestasis in which biliary bile acid levels are low. Preliminary studies indicate that some canalicular proteins, including GGT and carcinoembryonic antigen (CEA), are poorly expressed at the canaliculus in PFIC1 and 2. It is possible that low serum GGT levels result from the lack of canalicular GGT available for elution as well as from the inadequate concentrations of intracanalicular bile acids to act as detergents [51,52].

- *Serum transaminases*: In PFIC1 serum transaminases are mildly elevated. While in patients with PFIC2, serum transaminases levels are usually elevated to at least five times normal values. In PFIC3, serum aminotransferases, conjugated bilirubin, and ALP are all significantly elevated [2,45].
- *Serum cholesterol*: it is characteristically low or normal in all the three types [3].
- *Serum bile acid concentration*: it is elevated in all the three types [44,45].
- *Alpha-fetoprotein*: it is elevated at diagnosis in PFIC2 than that in PFIC1 [12,45].
- *Absent serum lipoprotein X (LPX) in PFIC3*: because measurement of biliary phospholipids is impractical in the evaluation of most patients, measurement of serum LPX may serve as a surrogate marker for PFIC3. LPX is the predominant lipoprotein in the plasma of cholestatic patients. LPX is absent from the serum of patients with homozygous *ABCB4* mutations. LPX is probably composed of biliary vesicles that are formed at the subapical compartment of the hepatocyte, transcytosed to sinusoidal membrane, and released into plasma. This process is absolutely dependent on MDR3, but the precise mechanism has not been defined [50,53].

2. Biliary bile analysis:

Biliary bile analysis is performed on gallbladder bile or on bile collected by duodenal aspiration (pure choledochal bile). In case of gallbladder puncture, bile contamination by blood may falsify bile analysis. In case of duodenal aspiration, bile dilution or bile contamination by alimentary phospholipids may falsify bile analysis [3].

The biliary bile salt concentration is dramatically decreased (<1 mmol/L) in PFIC2 patients [54] and only mildly decreased in PFIC1 patients (3–8 mmol/L) [19]. The normal concentration of biliary primary bile salts distinguishes PFIC3 patients from those with PFIC1 and PFIC2 [42].

In PFIC3 patients, the cardinal feature is the dramatically decreased biliary phospholipid level (1–15% of total biliary lipids; normal range 19–24%). Biliary bile salt-to-phospholipid is approximately 5-fold higher than in wild type bile, as is also biliary cholesterol-to-phospholipid [3].

3. *Radiological:*

Initial ultrasonography of the liver is performed to exclude biliary tract disease. Typically, ultrasonography is normal but may reveal a huge gallbladder in PFIC3. Sometimes, biliary stones may be identified in both PFIC2 and PFIC3 [3].

Cholangiography performed in a limited number of patients with PFIC3 showed a normal biliary tree, excluding sclerosing cholangitis, and allowed bile to be collected for biliary lipid analysis [42].

4. *Histopathology: Liver biopsy shows:*

- *In PFIC1*

Light microscopy (LM): on routine hematoxylin and eosin (H & E) staining, liver biopsy shows bland cholestasis with almost no inflammation. It shows canalicular bile plugs of distinctive color. Small-duct paucity may be present. Fibrosis starts early, with approximately 75 % of patients having some fibrosis by 2 years of age. Fibrosis may appear initially either as pericentral sclerosis or portal fibrosis, or sometimes both. Portal to central bridging then develops in association with lacy lobular fibrosis and eventually leads to cirrhosis. Proliferating bile ductules are observed at the edge of the portal tracts in patients with significant fibrosis. The rate of progression of the fibrosis is highly variable but correlates loosely with the severity of the clinical disease [45].

On electron microscopy (EM), canalicular bile plugs shows characteristic granular appearance “chunky bile”.

- *In PFIC2*

LM: on H & E stains, there is inflammation with giant cell hepatitis, fibrosis and duct reaction [45].

On EM, bile appears amorphous [45].

- *In PFIC3*

LM: on H & E stains, bile ductular proliferation and mixed inflammatory infiltrates are observed in the early stages despite patency of intra- and extrahepatic bile ducts. Cholestasis with slight giant cell transformation and isolated eosinophilic necrotic hepatocytes may also be present. Periductal sclerosis affecting the interlobular bile ducts eventually occurs. Extensive portal fibrosis evolves into biliary cirrhosis in older children [42].

EM of liver has not been reported in proven cases.

5. *Immunohistochemical staining:*

Commercially available MDR3 and BSEP antibodies allow liver immunostaining to be performed. Absence of canalicular or mild immunostaining is in favor of a gene defect. However, normal staining does not exclude a gene defect as a mutation may induce a loss of function but normal synthesis [31,42].

6. Genetic testing:

Molecular analysis remains the definitive diagnostic technique for PFIC. Gene analysis is usually performed by DNA sequencing of the 27 coding exons (coding exons 2-28) of the *ATP8B1*, *ABCB11*, and *ABCB4* genes and their splice junctions [3]. The use of a resequencing chip dedicated to genetic cholestasis could facilitate identification of gene mutation [55].

	PFIC-1	PFIC-2	PFIC-3
Synonyms	Byler disease ATP8B1 deficiency FIC1 deficiency	Byler syndrome ABCB11 deficiency BSEP deficiency	ABCB4 deficiency MDR3 deficiency
Gene defect	<i>ATP8B1 (FIC1)</i>	<i>ABCB11 (BSEP)</i>	<i>ABCB4 (MDR3)</i>
Locus	18q21-22	2q24	7q21
Transport defect	Aminophospholipid	Bile acid	Phospholipids
Pathophysiology	Impaired inward translocation of aminophospholipids over cellular membranes	Impaired canalicular bile salt transport secondary to malfunction of BSEP	Impaired canalicular translocation of phosphatidylcholine
Clinical picture:			
Onset of cholestasis	Neonatal	Neonatal	Variable
Pruritus	Severe	Severe	Moderate
Extrahepatic manifestations	Present	Absent	Absent
Cholelithiasis	Absent	Increased incidence	Increased incidence
HCC	No	Risk from the 1 st year	No
Clinical spectrum of gene defect	PFIC1, BRIC1, and ICP	PFIC2, BRIC2, and ICP	PFIC3, ICP, adult biliary cirrhosis, cholelithiasis, transient neonatal cholestasis, drug induced cholestasis
Biochemical:			
GGT	Normal or low	Normal or low	Elevated
Transaminases	Mildly elevated	More elevated	Elevated
Bile acids	Elevated	More elevated	Elevated
Cholesterol	Normal	Normal	Normal
Alpha fetoprotein	Not significantly elevated	More elevated	--
Histopathological	Bland cholestasis (LM) Coarse granular bile (EM)	Giant cell hepatitis (LM) Amorphous bile (EM)	Ductular proliferation (LM)
Immunohistochemical	--	Absent or reduced BSEP staining in the majority of patients	Absent or reduced MDR3 staining in about 50 % of patients

Table 1. Summary of the criteria of different PFIC types.

6. Differential diagnosis

Two groups of diseases are in differential diagnosis with PFIC group of disorders. For PFIC1 and PFIC2, it is to be differentiated from other cholestatic disorders with low GGT. While for PFIC3, when it presents early it, is to be differentiated from cholestatic disorders with high GGT and when it presents in an older age, childhood or adolescence, it is to be differentiated from other causes of chronic liver diseases at respective ages.

- *Cholestasis with low GGT:*
 1. Inborn errors of bile acid metabolism [6].
 2. Familial hypercholanemia: familial hypercholanemia represents a PFIC-like disorder due to a bile canalicular tight junction protein defect combined with a defect of primary bile acid conjugation. Cholestasis is due to impaired transport of unconjugated bile acids into bile and to bile leakage into plasma through abnormal canalicular tight junctions increasing paracellular permeability [56].
 3. Arthrogyrosis- renal dysfunction cholestasis (ARC) syndrome is a complex disease due to mutation of *VPS33B* involved in intracellular trafficking and targeting of apical proteins. The gene defect results in a loss of apical protein expression in the liver and kidneys [57].
- *Cholestasis with high GGT:*
 1. Biliary atresia [58].
 2. Neonatal sclerosing cholangitis [59].
 3. Congenital cytomegalovirus (CMV) infection.
 4. Alpha1-antitrypsin deficiency disease [60].
 5. North American Indian Childhood Cirrhosis (NAIC) [61].
 6. Aagaens syndrome (hereditary cholestasis with lymphedema): a very rare familial cholestatic disorder with cholestasis and lower limb edema [62].
- *Causes of chronic liver disease:*
 1. Chronic viral hepatitis.
 2. Autoimmune liver diseases: autoimmune hepatitis and autoimmune sclerosing cholangitis.
 3. Metabolic liver disorders, e.g., Wilson disease and alpha1-antitrypsin deficiency.

7. Treatment

Initial treatment of PFIC includes the use of cholestyramine, ursodeoxycholic acid, rifampicin, and phenobarbital [63-65]. Until the late 1980s, liver transplantation was the only effec-

tive therapy for those who did not respond to medical treatment [66,67]. Later on, less invasive non-transplant surgical approaches were proposed and undertaken early in the course of the disease with promising initial results [68]. In this section, a brief overview about the different lines of management for PFIC patients will be given.

7.1. Medical therapy

Unfortunately, most forms of medical therapy for PFIC types 1, 2, and 3 are of limited effectiveness. Nevertheless, several treatment modalities can be used in specific patients to improve quality of life or prevent progression of the disease [2,69].

- *Cholestyramine* is an anion-exchange resin that binds bile salts, preventing their re-absorption in the enterohepatic circulation. In PFIC, relief of pruritus and normalization of biochemical parameters is only described rarely with cholestyramine. However, in patients with BRIC it can be helpful in shortening episodes [2].
- *Rifampicin*, although it accelerates the hepatic detoxification and excretion of compounds, such as bilirubin and bile salts, it has been used with limited efficacy in patients with PFIC [51]. Nevertheless, in patients with BRIC it can completely abort an episode.
- *Ursodeoxycholic acid (UDCA)* is a relatively hydrophilic bile salt, which is less cytotoxic than endogenous bile salts. Upon oral administration (20 mg/kg/day), it will partially replace endogenous bile salts in the bile salt pool, reducing injury of the hepatocytes during cholestasis. In PFIC3 regular administration of UDCA normalizes liver function tests and improves clinical parameters in up to 50% of the patients. The therapeutic effect appears to be dependent on the type of mutation, with premature stop codons leading to a truncated protein being associated with nearly no response to therapy. UDCA should therefore be the first choice in the initial therapeutic management of patients with ABCB4 deficiency, especially when a missense mutation in the corresponding gene is found [42]. In patients with PFIC1 or PFIC2, the results of UDCA treatment are conflicting, ranging from clear improvement to no effect at all.

In this respect, the recommended treatment strategy is to start with UDCA therapy in all types of PFIC, especially PFIC3. If no appropriate response, especially regarding pruritus, add the other medical lines of therapy. Those who will not respond are shifted to surgical treatment [3,45,65,66].

7.2. Surgical treatment

Surgical treatment for PFIC is an important major line of therapy. If no complete clinical or biochemical improvement is obtained with medical therapy, more invasive therapy such as biliary diversion or even liver transplantation is necessary [2,70].

Interruption of the enterohepatic circulation through biliary diversion has yielded excellent clinical, biochemical, and histologic response in a number of children with PFIC, provided the procedure is performed before the development of significant hepatic fibrosis [46]. It reduces

the accumulation of toxic bile salts by decreasing their intestinal re-uptake. It is unclear if these approaches are optimal for specific genetic forms of PFIC rather than others. It is possible that these interventions may be best for severe PFIC1 and milder phenotypic variants of PFIC2. Nasobiliary drainage may help to select potential responders to biliary diversion [71].

There are three major non-transplant surgical techniques to permanently interrupt the enterohepatic circulation, namely partial external biliary diversion (PEBD), ileal bypass (IB) and partial internal biliary diversion (PIBD).

- *Partial external biliary diversion (PEBD):*

PEBD interrupts the enterohepatic circulation of bile salts by partially diverting bile from the gallbladder through a loop of jejunum connecting the gallbladder to the abdominal skin [72].

In 1988, Whittington and Whittington [68] introduced cholecystojejunocutaneostomy as a PEBD for the surgical treatment of PFIC, to increase the elimination of bile acids accumulated within the body and thus control the intractable pruritus. In this procedure, one end of a loop of jejunum is anastomosed to the dome of the gallbladder, whereas the other is used to form a cutaneous ostomy (Figure 2A). Bile in the gallbladder then flows either out of the ostomy or into the intestine. Typically 30–50% of bile drains out of the ostomy and is discarded. Two variants on the original PEBD have also been described; one using a laparoscopic technique [73] and the other using an appendiceal conduit [74].

Results of PEBD are promising with respect to pruritus, jaundice and histology, both in patients with PFIC1 and PFIC2, with at least partial improvement in more than 75% of the patients [66,72]. Although this seems promising, at present it is unclear whether in patients responding to PEBD liver transplantation can also be avoided at long-term follow-up [75]. Moreover, some patients do not benefit from biliary surgery at all. Obviously in these patients liver transplantation should be considered [72].

The type of mutation seems to be associated with the outcome of PEBD, with better prognosis in disease caused by milder mutations, especially for the ABCB11 mutations E297G and D482G [32,45]. However, when severe fibrosis is already present at the moment of PEBD, prognosis is worse [72]. One patient with PFIC3 who underwent PEBD was described in literature; this patient showed no improvement [67].

No serious PEBD complications are reported, although problems with the stoma (stenosis, recurrent bleeding) (Figure 2) sometimes make a re-operation necessary. In addition excessive stomal losses can cause dehydration and electrolyte imbalance, while cholangitis can also develop [72,75].

The permanent character of the PEBD makes it less suitable for patients with episodic cholestasis (BRIC). In these patients temporary nasobiliary drainage (NBD) to interrupt the enterohepatic circulation can be endoscopically introduced. This procedure is effective in most of these patients, resolving pruritus and normalising bile salts within short time [10,71].

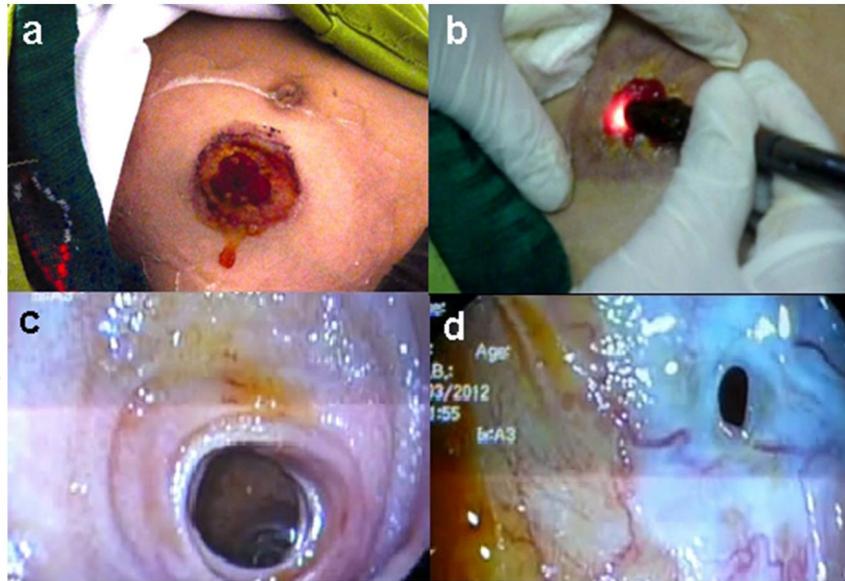


Figure 2. A seven years old child diagnosed as PFIC2 underwent PEBD at the age of 2 years old with good outcome. He had recurrent bleeding from the ostomy opening (a). Endoscopy through the ostomy opening (b) showed free jejunal loop (c) till its proximal end at the gall bladder (d). The source of bleeding was the stoma itself.

- *Terminal ileal exclusion or ileal bypass (IB):*

Although most of PFIC patients and their parents tolerate well PEBD with its external biliary fistula and the need for stoma care, sometimes it becomes a real problem, particularly for children of school age and teenagers, who may feel uncomfortable to participate in all activities with their friends. Moreover, there is still a group of patients who cannot undergo PEBD because of a previous cholecystectomy, or who develop postoperative electrolyte imbalance due to the excessive daily amount of bile [5,76].

To deal with these problems, an IB technique was proposed. In IB, the terminal ileum is skipped by an ileocolonic anastomosis. It was developed as an alternative treatment to PEBD, that avoids a long-term stoma complications. In 1994, Whittington et al. described a good initial outcome of IB in two patients after cholecystectomy, but a chronic diarrhea occurred one year later. In 1998, Holland et al. described this procedure in PFIC children after cholecystectomy. All patients were supplemented with vitamin B12 and folic acid. Interestingly, no diarrhea was reported postoperatively. Early results were very promising, with a relief of pruritus and normalization of bilirubin level. Nevertheless, relapse of cholestasis occurred in half of the patients. The authors underline that IB is not as effective as PEBD and therefore it should not be considered as the primary treatment in children with PFIC [5,51].

The rationale of this technique was that the vast majority of intestinal bile salts are reabsorbed in the distal ileum; that is the distal 15% of the small intestine. Therefore, exclusion of this segment of intestine may lead to bile acid wasting. The small intestine is transected at a point that demarcates the distal 15% of the small intestine, and a blind loop is formed with the distal ileal segment. The proximal loop of the intestine is sewn end-to-side to the cecum, completing the internal bypass of the distal ileum. Accurate assessment of the appropriate

amount of ileum for bypass is likely to be critical; too little is unlikely to be therapeutic and too much is likely to yield bile acid-induced diarrhea. Mutational analysis may be used eventually to predict which patients are most likely to benefit from surgery. After IB, symptoms may recur within one year requiring conversion to PEBD [5,77].

- *Partial internal biliary diversion (PIBD):*

PIBD interrupts the enterohepatic circulation of bile salts by partially diverting bile from the gallbladder through a loop of jejunum connecting the gallbladder to the colon [46,76].

This operation combines the advantages of partially diverting the biliary flow from the enterohepatic cycle (such as the PEBD does), while at the same time avoiding an external biliary fistula. In addition, this operation lacks the potential for malabsorption that may result from partially excluding the terminal ileum from the intestinal transit. There is, however, a potential for choleric diarrhea, which may result from large amounts of bile salts entering the colon. Because of this, it was strongly emphasized that the conduit should be made at least 15 cm long to create a certain resistance to the bile flow; it is believed that this stimulates a certain amount of bile to flow through the normal biliary tract to the duodenum. This problem occurred in a transient way in a few of the patients and it can be controlled with the use of cholestyramine for a limited span of time [46].

Through an upper midline abdominal incision, the gallbladder and the liver are evaluated. An intestinal conduit is constructed using a 15- to 20-cm segment of midjejunum, which is sutured initially to the gallbladder wall and then terminolaterally to the midportion of the ascending colon. The distal end of the jejunum is slightly tapered in a way that the jejunum could reach the colon in an isoperistaltic direction to prevent colonic contents from entering the conduit. The clinical and laboratory results described for PIBD make it a very attractive surgical option for the treatment of PFICs in children with a normal gallbladder. However, long-term follow-up is necessary to evaluate late results and eventual complications associated with this technique [46].

If all previously described therapies fails in controlling pruritus, when there is an end-stage PFIC liver disease, or when the disease is progressive despite treatment; orthotopic liver transplantation (OLT) remains the only alternative [78-80]. Before the development of liver transplantation, therapy for these patients was generally ineffective. With the advent of liver transplantation, many PFIC patients were treated with this life-saving procedure [70,78]. At one time, PFIC was among the 5 most common indications for liver transplantation in children [81,82].

Although OLT is associated with serious surgical risks and lifetime immunosuppressive therapy is necessary, it usually gives complete correction of phenotype in patients with PFIC2 and PFIC3 deficiency in which the disease is hepatocyte specific. However, phenotypic recurrence of severe PFIC2 deficiency post-transplantation can occur as a result of the formation of autoantibodies against BSEP [83,84]. Intensifying immunosuppressive therapy may resolve this problem.

In contrast, in PFIC1, liver transplantation is potentially fraught with a number of potential complications related to the extrahepatic expression of the *ATP8B1* gene. The most prominent posttransplantation problems include intractable diarrhea, hepatic steatosis, poor growth, and recurrent pancreatitis. Worsening diarrhea post liver transplant might be due to an imbalance between bile salt excretion and re-absorption, since the hepatic graft excretes a normal amount of bile salts, whereas the intestine remains functionally impaired. The resulting increased amount of bile salts in the ileum and colon induces or worsens diarrhea, which might respond to cholestyramine treatment [19,85]. Therefore, in PFIC1, non-transplant surgical approaches should be considered the preferred first-line of therapy.

In summary, children with PFIC do better with non-transplant surgical interventions than they do with the natural history of disease, which is uniformly fatal. Successful outcomes have been demonstrated, with marked improvements in clinical symptoms, laboratory values, growth and histology. It appears that the success rate is high enough that many patients may do better with a non-transplant procedure than transplant given the posttransplant morbidities associated with immunotherapy. Those with more advanced disease are most likely to have a poor outcome with non-transplant surgical procedures. This may encourage clinicians to consider a surgical intervention early in the course of disease before significant hepatic scarring develops [76].

Some authors proposed that the treatment strategy is to perform PEBD rapidly after diagnosis in patients with PFIC1 & PFIC2 and to consider OLT when treatment fails. In patients with PFIC3, UDCA treatment is the first-line therapy; if not successful it is followed by liver transplantation. In patients with episodic cholestasis (BRIC) medical treatment with rifampicin with or without cholestyramine can be attempted at the start of an attack. If medication is not successful in aborting the cholestatic episode NBD can be performed [2,69]. In BRIC patients who progress to a more permanent form of cholestasis, or in patients with very frequent or debilitating attacks, a biliary diversion can be considered [69].

7.3. New and future therapies

New and future therapies for PFIC patients include hepatocyte transplantation, the use of nuclear receptor ligands, enhancing the expression of the mutated transporter protein by employing chaperones and mutation specific therapy [2,69].

Hepatocyte transplantation has been successful in partially repopulating the liver, diminishing pathology in a mouse model of *ABCB4* deficiency, but unfortunately not yet in patients [86]. In *ABCB11* deficiency it is doubtful whether hepatocyte transplantation is a good therapeutic option since possible premalignant cells are left in place.

Certain nuclear receptors regulate bile formation. The key nuclear receptor in bile formation is the bile salt sensor FXR. Activated FXR transactivates a number of genes, resulting in improved bile salt excretion and detoxification. Targeting FXR with synthetic ligands is explored as a possible therapeutic option for cholestasis syndromes [87].

A pharmacological chaperone is defined as a small molecule that specifically binds to its target protein and induces or promotes proper folding and trafficking of the protein [88]. Some

researchers have investigated the usefulness of pharmacological chaperones for treatment of diseases caused by folding-defective membrane proteins [24,89,90]. Pharmacological chaperones such as 4-phenylbutyrate acid (4-PBA) have been shown to stabilize proteins misfolded due to missense mutations, thereby preventing degradation in the endoplasmic reticulum. In vitro, 4-PBA enhances cell surface protein expression for some of the missense mutations found in ATP8B1 deficiency and ABCB11 deficiency [25,91]. Misawa et al [24], showed that bile acids do act as pharmacological chaperones of E297G BSEP. They also described the discovery and structural development of non-steroidal compounds with potent pharmacological chaperone activity for E297G BSEP.

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