Progressive Familial Intrahepatic Cholestasis

Definition
Maintenance of normal bile flow is dependent in part upon a complex system of specific membrane proteins that are found in a polarized distribution in the liver and intestine. Inherited defects in the genes for some of these transporters lead to cholestasis, which can result in a clinical syndrome generally referred to as Progressive Familial Intrahepatic Cholestasis (PFIC). PFIC was initially described as a clinical rather than a genetic entity. In its most characteristic form, PFIC involved cholestasis presenting in the first year of life. The cholestasis was persistent and led to progressive liver injury. Imaging and other invasive studies did not reveal evidence of extrahepatic bile duct obstruction or disease – thus the label of intrahepatic disease. In many of the early descriptions, the disease was identified in multiple relatives, hence the term familial. There is a broad spectrum of disease in PFIC, ranging from mild to severe, depending on the specific gene defect present.

The best-understood forms of PFIC involve inherited defects in three specific genes (Table 1) and are the subject of current investigation by ChiLDREn. Rapid advances in this field have identified new genetic etiologies for PFIC (e.g tight junction protein 2 below) and there are likely other genes involved in the spectrum of disease in PFIC, which may be implicated in the future.

**FIC1 Deficiency.** PFIC1 denotes individuals who have defects in the Familial Intrahepatic Cholestasis 1 gene (FIC1 = ATP8B1), which cause progressive disease. This disease was initially described as two distinct clinical entities, Byler disease and Benign Recurrent Intrahepatic Cholestasis (BRIC). Both diseases are the result of abnormalities in FIC1. Current thinking is that the diseases vary due to differing severity of the underlying defect in FIC1, with milder defects being present in BRIC. It is likely that variations in other genes modify the FIC1 phenotype (See references below). FIC1 mediates the flipping of aminophospholipids from the outer to inner hemi-leaflet of the canalicular lipid bi-layer. The exact nature of how FIC1 deficiency causes disease is not known. Some studies indicate that FIC1 may influence the expression of bile acid transporters via effects on the transcription factor, farnesoid X receptor. Other studies indicate that FIC1 may alter the composition of membranes and therefore alter transporter function.

**BSEP Deficiency.** PFIC2 was initially described in children with low ggt cholestasis that could not be ascribed to defects in FIC1. Gene linkage and basic investigations of bile acid transport led to the discovery that PFIC2
was the result of defects in the canicular bile salt export pump (BSEP = ABCB11). BRIC-like disease (BRIC2) and Intrahepatic Cholestasis of Pregnancy (ICP) have been described in children and adults with genetic defects in BSEP. BSEP plays a critical role in transporting bile acids from inside the hepatocyte into the bile canaliculus and thus it is not surprising that inherited defects in this gene lead to cholestatic liver disease.

**MDR3 Deficiency.** PFIC3 was initially discovered in knock-out mice and then identified in children with a distinct form of PFIC, characterized by high serum levels of gamma glutamyl transpeptidase activity (gGTP). The elevation in serum gGTP was in distinct contrast to the normal or low levels of gGTP that characterize the prior two forms of PFIC. The underlying gene that is defective in PFIC3 encodes multidrug resistance-associated protein 3 (MDR3 =ABCB4). MDR3 mediates the flopping of phospholipids from the inner to outer hemi-leaflet of the canalicular lipid bi-layer, thereby facilitating excretion of phospholipids. ABCB4 mutations have also been associated with low phospholipid associated cholelithiasis syndrome and ICP.

The nomenclature for these diseases is in part historical and confusing in light of molecular advances in our understanding of PFIC as a whole. Going forward, ChiLDREN has agreed to designate these diseases on the basis of the names of the defective protein that underlies the disease. Thus PFIC 1, 2, and 3 will henceforth referred to as FIC1, BSEP, and MDR3 deficiency, respectively.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Proposed Function, substrate</th>
<th>Common Disease Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP8B1</td>
<td>FIC1</td>
<td>P-type ATPase; aminophospholipid flippase at the canalicular membrane (a translocase that transports aminophospholipids from outer to inner layer)</td>
<td>PFIC1 (Byler Disease), BRIC1</td>
</tr>
<tr>
<td>ABCB11</td>
<td>BSEP</td>
<td>Canalicular protein with ATP binding cassette (ABC family of proteins); works as a pump transporting bile acids across the canalicular membrane of the hepatocyte into the canaliculus</td>
<td>PFIC2, BRIC2 ICP</td>
</tr>
<tr>
<td>ABCB4</td>
<td>MDR3</td>
<td>Canalicular protein with ATP binding cassette (ABC family of proteins); works as a phospholipid floppase at the canalicular</td>
<td>PFIC3, ICP, Cholelithiasis</td>
</tr>
</tbody>
</table>
membrane (a translocase that transports phospholipid from inner to outer layer)

Table 1
Abbreviations: PFIC – progressive familial intrahepatic cholestasis, BRIC – benign recurrent intrahepatic cholestasis, ICP – intrahepatic cholestasis of pregnancy

Tight Junction Protein 2 (TJP2) Mutation

Recently, a cohort of 12 patients has been described with the clinical picture of PFIC but absent an identifiable mutation. Whole genome sequencing identified a protein truncating mutation in tight junction protein 2 (TJP2). TJP2 is an important cytosolic component of cell-cell junctions. All individuals with the TJP2 mutation were noted to have severe liver disease with the majority requiring liver transplantation. Evaluation of the implications of this disease causing mutation promises to be an exciting avenue of future investigation.

Liver Disease

Liver disease in PFIC results from the effects of hepatocellular accumulation of bile acids. The liver disease may be mild or severe, depending on the specific gene defect present. Intracellular accumulation of bile acids can lead to liver injury by a number of mechanisms including both direct toxicity and pathologic activation of signal transduction pathways. In FIC1 deficiency biliary excretion of bile acids is diminished, potentially as a result of decreased although not absent expression or function of BSEP. Other factors, currently under investigation, are also likely to play a role in the pathogenesis of the cholestasis in FIC1 disease.

Since interruption of canalicular excretion of bile acids is not absolute, the rate of progression to end-stage liver disease in FIC1 deficiency may be slower than in BSEP deficiency. In severe forms of BSEP deficiency, BSEP expression and function are completely absent. Hepatocellular bile acid excretion can only occur through alternative and quantitatively insufficient pathways. Hepatocellular bile acids accumulation is pronounced causing rapidly progressive liver disease. End-stage liver disease in severe BSEP deficiency can occur in the first one to two years of life. The pathogenesis of liver disease in MDR3 deficiency is different from the other two forms of PFIC. In MDR3 deficiency, phospholipids in canalicular bile are either deficient or absent leading to the formation of a toxic bile rich in unmicellized bile salts and contributing to the pathogenesis of a progressive intrahepatic cholangiopathy. The resulting liver disease is a consequence of the
cholestasis and inflammatory response generated by this cholangiopathy. In addition, hepatocellular injury in MDR3 deficiency results from hepatocellular bile salt accumulation.

**Clinical Features**

The typical presenting clinical features of the severe forms of these diseases are jaundice and/or pruritus. Life-threatening hemorrhage, secondary to cholestasis-related vitamin K deficiency, can also be a dramatic early presentation of PFIC. Genetic advances have allowed identification of individuals with less severe mutations in these genes and there is a growing appreciation that the clinical spectrum of disease in PFIC is quite diverse.

Profound, medical therapy-resistant pruritus is one of the most common early manifestations of all three of these forms of PFIC. Irritability may be an early manifestation of pruritus in infants who cannot scratch. Typically scratching begins between 6 and 12 months of age. The scratching is constant and has profound effects on quality of life for both the patient and family. Many children may not have jaundice and the pruritus is incorrectly ascribed to atopy or dermatitis.

The initial laboratory findings in children with PFIC can make identification of liver disease problematic. The cholestasis in these children is characterized by marked elevations in serum bile acid levels. This can be in the setting of near normal serum bilirubin, normal gGTP, normal serum cholesterol and only mild elevation in serum aminotransferase values. Since serum bile acid concentrations are not routinely measured, it may initially be difficult to appreciate that these children have significant cholestasis. In MDR3 deficiency gGTP is elevated. As liver disease in these children progresses, the biochemical parameters become more typical for chronic liver disease and can include elevated bilirubin and aminotransferase values.

The slower clinical progression of FIC1 disease may help distinguish it from severe forms of BSEP. At presentation, patients with BSEP deficiency have higher serum aminotransferase, bile salt, albumin and alpha-fetoprotein levels, and lower alkaline phosphatase values, than do FIC1 disease patients; BSEP deficiency patients are also more likely to have elevated white blood cell counts. Patients with BSEP deficiency are more likely to demonstrate multi-nucleated giant cells at liver biopsy, negative staining for BSEP upon liver immunohistochemistry, and are more prone to gallstone disease, portal hypertension, hepatocellular carcinoma, and early liver failure. In contrast, patients with FIC1 disease tend to have more extra-hepatic symptoms (e.g. sensorineural hearing loss). Exocrine pancreatic function and serum pancreatic
enzymes are normal in patients with BSEP deficiency.

The cholestasis in PFIC is associated with malabsorption of fat and fat-soluble vitamins. Thus failure to thrive is a common early feature of disease that results from malabsorption of long chain fats found in breast milk and many commercial infant formulas. Complications of fat-soluble vitamin deficiencies (A, D, E and K) can also be seen. Hemorrhage secondary to vitamin K deficiency and rickets from vitamin D deficiency are the most dramatic and acute problems. Long-term complications of deficiencies of the other fat-soluble vitamins are well described and include neuropathy and visual problems.

Children with PFIC develop end-stage liver disease in a manner akin to other forms of progressive cholestatic liver disease. Portal hypertension develops secondary to the development of biliary cirrhosis. All of the typical sequelae of portal hypertension have been described in PFIC, including growth failure, ascites, and variceal hemorrhage. BSEP deficiency has a strong association with hepatocellular carcinoma. Other pancreatico-biliary malignancies have been described as well in BSEP deficiency. Synthetic liver failure is a late manifestation of these diseases.

**Diagnosis**

Definitive diagnosis of a specific form of PFIC is dependent upon identification of characteristic genetic defects. Surrogate markers may be used inform the diagnosis, however gene tests are confirmatory. The typical patient with either FIC1 or BSEP deficiency has profound symptomatic cholestasis (as documented by marked elevation of serum bile acids) with normal or low serum levels of gGTP. Recent studies suggest that at presentation, serum aminotransferases, bile salt levels and serum alkaline phosphatase are higher in BSEP deficiency, while serum albumin tends to be lower in FIC1 deficiency. At present, it is not certain if one can readily distinguish FIC1 from BSEP deficiency by surrogate testing, and with some forms of treatment distinction may not be essential. Assessment of risk of malignancy and probable response to liver transplantation (see below for treatment and prognosis) are features that likely are strongly disease-associated. A lack of canalicular staining for BSEP, or MDR3 is highly suggestive of BSEP deficiency and MDR3 deficiency, respectively. FIC1 deficiency is systemic and thus certain non-hepatic features suggest FIC1 deficiency rather than BSEP deficiency. Children with FIC1 deficiency may have somatic growth problems, hearing problems, recurrent respiratory problems, elevated sweat chloride, recurrent pancreatitis, diarrhea that is independent of the cholestasis, post-transplant steatosis with possible progressive disease and cirrhosis secondary to steatohepatitis, and intractable diarrhea. BSEP deficiency appears to be a liver-specific
disease and may be associated with an increased risk of liver cancer. BRIC related to defects in BSEP may be associated with cholelithiasis. Early on, FIC1 deficiency appears to cause relatively less hepatocellular injury than BSEP deficiency. Retrospective studies have demonstrated that progression to end-stage liver disease occurs more quickly in BSEP deficiency, although this impression needs to be confirmed in prospective analyses of the clinical course of children with genetically defined disease. The advent of commercially available genetic testing provides a standard for definitive diagnosis in PFIC. Available lab testing for the individual genes may be found and referenced at www.genetests.org.

MDR3 deficiency should be suspected in children with progressive cholestasis or chronic unexplained liver disease who have an elevated gGTP and no evidence of extrahepatic bile duct disease. In patients with severe disease, biliary phospholipid concentrations are markedly reduced and there may be an absence of serum lipoprotein X. Some individuals with partial defects in MDR3 have more subtle hepatic presentations. This may include intrahepatic cholestasis of pregnancy, drug-induced cholestasis and a form of benign recurrent intrahepatic cholestasis. Low phospholipids-associated cholestasis syndrome is caused by a mutation in MDR3 and presents as cholesterol gallstones and intrahepatic cholelithiasis in adults younger than 40 years. At present, without genetic testing, it is not possible to make a definitive diagnosis of MDR3 deficiency.

Histologic and ultrastructural analysis of the liver may be useful in distinguishing FIC1 from BSEP deficiency. Hepatocytes in FIC1 deficiency tend to be tidy and compact (FIC1 H and E – image 2) with the major observed abnormality being bland intracanalicular cholestasis. In contrast, BSEP deficiency is histologically associated with more pronounced hepatocellular disarray, edema, giant-cell change, and hepatocellular necrosis ("neonatal hepatitis") in BSEP deficiency (BSEP H and E – Image 1) than in FIC1 deficiency. Additionally, portal and lobular fibrosis is more often seen at presentation in BSEP deficiency, but both FIC1 and BSEP deficiencies can progressively develop increasing amounts of fibrosis and a subset result in cirrhosis. Severe BSEP deficiency also is associated with a lack of demonstrable immunohistochemical stain for BSEP along the canaliculi (BSEP immunohistochemistry 1 – disease – Image 3 and BSEP immunohistochemistry 2 – control – Image 4) and BSEP immunohistochemistry BSEP deficiency – Image 5) although molecules of similar structure, such as multiple drug resistance protein 2 (MRP2), are normally expressed along canalici in patients with severe BSEP deficiency (MRP2 immunohistochemistry BSEP deficiency – Image 5) (Legend - Anti-MRP2 antibody / hematoxylin, 200 x). Transmission electron microscopy
in FIC1 deficiency may identify coarsely granular "Byler bile" which is relatively specific for this disorder (FIC1 deficiency canalicular ultrastructure – Image 6): {Legend -- "FIC1 deficiency, canalicular bile, 40,000 x"}, while transmission electron microscopy in BSEP deficiency may identify a more non-specific loose, amorphous, or dense bile (BSEP deficiency canalicular ultrastructure – Image 7: {Legend -- "BSEP deficiency, canalicular bile, 42,625 x"}). MDR3 deficiency can display expanded portal tracts and ductular proliferation with mixed inflammatory infiltrate mimicking a biliary obstruction pattern of injury or extensive portal fibrosis and biliary cirrhosis. Lobular cholestasis and giant cell transformation may also be present. Absent canalicular immunohistochemical staining for MDR3 is highly suggestive, although not pathognomonic for MDR3 deficiency.

**Treatment**
The treatment of PFIC includes standard nutritional approaches for fat and fat-soluble vitamin malabsorption due to cholestasis and therapies for end-stage liver disease. Certain aspects of the management of the cholestasis in PFIC are unique and are described here. Initially, the pruritus associated with PFIC is the most prominent and debilitating symptom. Standard medical approaches (e.g., ursodeoxycholic acid, cholestyramine, rifampin, and opioid antagonists) to the pruritus are minimally or transiently successful if at all. Surgical interruption of the enterohepatic circulation of bile acids can be a very effective therapy in children with PFIC. Nasobiliary drainage of bile may accomplish the same thing on a temporary basis, has been used in adults with BRIC and may be helpful in assessing response to treatment. These procedures can ameliorate the pruritus, normalize serum markers of liver disease, and prevent progression of liver disease. The exact mechanism by which this works is not known. The most commonly used surgical procedure for PFIC is partial external biliary diversion. This involves using a small segment of intestine to form a conduit between the gallbladder and abdominal wall. Using this approach, 30 to 50% of bile excreted by the liver drains externally through the ostomy and is discarded. Modifications of this approach involve the use of a button device permitting intermittent drainage of bile and diversion to the colon or urinary bladder. The safety and efficacy of these modifications are not well understood. An alternative and less well-characterized approach for interrupting the enterohepatic circulation of bile acids involves partial ileal exclusion. A blind loop is formed with the distal 15% of the small intestine and the proximal limb of the intestine is anastomosed to the cecum. This bypasses the terminal ileum, where most bile acids are reabsorbed. These procedures were initially described for children with low-gGTP forms of PFIC. Some data suggests that biliary diversion is effective in FIC1 deficiency and mild to moderate BSEP deficiency and may not be effective in severe BSEP deficiency. Interruption of the enterohepatic circulation of bile acids may also be effective for other forms of
intrahepatic cholestasis, namely Alagille syndrome. Clinical improvement with normalization of serum bile acids within 1 year was associated with an excellent long-term outcome in patients with PEBD. The presence of cirrhosis at the time of PEBD indicates an increased chance of an unfavorable outcome. No studies have demonstrated a superiority of one type of non transplant surgical intervention to another, although there is a suggestion of a more durable response to biliary diversion compared to ileal exclusion. The risk for the development of hepatocellular carcinoma in BSEP deficiency may warrant bi-annual surveillance by ultrasonography and quarterly serum measurements of alpha-fetoprotein even after a “successful” biliary diversion procedure. Oral administration of ursodeoxycholic acid represents an effective therapy in milder cases of MDR3 deficiency with many patients normalizing liver function with therapy. Ursodeoxycholic acid is successful in resolution of gallstones in adult patients with low phospholipid associated cholelithiasis syndrome.

Prognosis
The prognosis for children with PFIC can be quite variable and influenced by the genetic abnormality (both the specific gene mutated and the severity of the mutation) and the therapeutic approaches used. Complete analyses of genotype and clinical course are not yet available so these statements must be viewed as preliminary. Severe defects in BSEP, especially those that are associated with a complete absence of protein or function, are associated with a high risk of hepatocellular carcinoma.

Transplantation
Severe defects in BSEP and MDR3 deficiency are typically associated with an unremitting form of cholestasis that is minimally if at all responsive to medical and surgical therapies, short of liver transplantation. End-stage liver disease typically evolves in the first five to ten years of life. Liver transplantation should be considered in MDR3 deficiency if there is no response to ursodeoxycholic acid therapy. Liver transplantation appears to be “curative” for MDR3 as the disease appears to be primarily liver specific. Recently, series of patients have been described in which “recurrent” BSEP deficiency has occurred after liver transplant. The “recurrent” disease is a manifestation of the development of an immune response toward the BSEP protein, which is a foreign protein introduced with liver transplantation. The liver biopsies in these patients showed canalicular cholestasis, giant cell transformation of hepatocytes, and slight lobular fibrosis, without evidence of rejection or biliary complications. Remission of these episodes may be achieved by specific immunosuppressive regimen directed toward BSEP antibody production (e.g. exchange transfusion, IVIG and rituximab).

In contrast to BSEP and MDR3, FIC1 defects lead to multisystem disease, which is expected in light of the
wide-spread tissue distribution of the FIC1 gene product. Liver transplantation in children with FIC1 deficiency has unmasked interesting and difficult issues in the post-transplant course. The most notable and incapacitating problem has been the development of intractable diarrhea. While not seen in most patients, this can be especially problematic when it occurs. Steatosis commonly develops in the liver graft and can be progressive leading to cirrhosis within years of liver transplantation. In FIC1 patients, external biliary diversion in the post-transplant period has been successful in reducing graft steatosis and resolving post transplant diarrhea and malabsorption. Recurrent pancreatitis is also problematic for some children after liver transplantation. Patient’s growth improves, however growth problems may not fully resolve in children with FIC1 deficiency after liver transplantation. Thus liver transplantation, while effective for pruritus, may not be an optimal therapy for children with FIC1 deficiency. Surgical interruption of the enterohepatic circulation appears to be preferable to liver transplantation in FIC1 deficiency. Overall, with optimal surgical intervention the long-term prognosis for children with PFIC is good.

ChiLDReN Network studies that include patients with PFIC
The ChiLDReN Network currently has one study that includes patients with PFIC.

The LOGIC study is a natural history study that includes patients with PFIC and three other rare liver diseases. A natural history study is aimed at acquiring information and data that will provide a better understanding of rare conditions. Participants will be asked to allow study personnel to obtain information from medical records and an interview, and to collect blood, urine, and tissue samples when clinically indicated, in order to understand the causes of these diseases and to improve the diagnosis and treatment of children with these diseases. All of the information obtained in these studies is confidential and no names or identifying information are used in the study.

LOGIC: A longitudinal study of genetic causes of intrahepatic cholestasis.
Eligibility: Children and adults ages 6 months through 25 years diagnosed with Alagille Syndrome, alpha-1 antitrypsin deficiency, progressive familial intrahepatic cholestasis, or bile acid synthesis defects, both before and after liver transplantation.
ClinicalTrials.gov Study NCT00571272

Organizations or foundations that help families dealing with PFIC
The ChiLDReN Network works with numerous groups that support patients and families who are dealing with
rare liver diseases. Please click here to go to that page on our website (Information for Families). You will see the list of groups and information about them.

**General Reviews**


- 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;31:3-10

**Clinical/Biochemical/Histology/Diagnosis**


Gastroenterol. 2008 Oct 20;8:47


Treatment


**FIC1 deficiency**


Frankenberg T, Miloh T, Chen FY, Anantharayanan M, Sun AQ, Balasubramaniyan N, Arias I, Setchell KD, Suchy FJ, Shneider BL. The membrane protein ATPase class I type 8B member 1 signals through protein kinase C zeta to activate the farnesoid X receptor. Hepatology. 2008 Dec;48(6):1896-905


Chen F, Ellis E, Strom SC, Shneider BL. ATPase Class I Type 8B Member 1 and protein kinase C zeta induce the expression of the canalicular bile salt export pump in human hepatocytes. Pediatr Res. 2010 Feb;67(2):183-7


**BSEP deficiency**


MDR3 deficiency


TJP2 Mutations