Review

Genetic basis of progressive familial intrahepatic cholestasis

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Progressive familial intrahepatic cholestasis (PFIC), originally known as Byler disease, was first described in an Amish kindred (1,2). It is an inherited disorder of childhood in which cholestasis of hepatocellular origin often presents in the neonatal period or the first year of life and leads to death from liver failure at ages ranging from infancy to adolescence. Cholangiograms show normal extra- and intrahepatic bile ducts. The pattern of appearance of affected children within families is consistent with autosomal recessive inheritance. On the basis of clinical, biochemical and histological features, several studies have provided support for the heterogeneity of this clinical entity, suggesting the existence of different types due to different disorders affecting the hepatocyte and related to a defect of bile secretion or bile acid metabolism (Table 1) (3–10). Recent molecular and genetic studies have allowed the identification of genes responsible for three types of PFIC (11–16) and have shown that PFIC was related to mutations in hepatocellular transport system genes involved in bile formation (Fig. 1) (17). These findings now provide specific diagnostic tools for the investigation of children with PFIC. This review summarizes the molecular defects that are associated with PFIC. Liver diseases resembling PFIC that have recently been identified as inborn errors in primary bile acid synthesis will not be considered (9,10).

Progressive Familial Intrahepatic Cholestasis, Type 1 (PFIC1, Byler’s Disease)

Children affected with this type of PFIC were originally described in an Amish family, the Byler family (1,2). PFIC1 is characterized initially by recurrent episodes of jaundice which becomes permanent later in the course of the disease, severe pruritus, normal serum gamma-glutamyltransferase activity and cholesterol level, high concentrations of serum primary bile acids and low biliary primary bile acid concentrations mainly affecting the biliary concentration of chenodeoxycholic acid, but low concentrations of chenodeoxycholic acid have also been found in bile of non-Amish patients. Liver histology in the first months of life shows canalicular cholestasis, minimal giant cell transformation and slight lobular and portal fibrosis with absence of a true ductular proliferation (1–3,5–9,18,19). Indeed, cytokeratin 19 immunostaining usually shows only a periportal biliary metaplasia of hepatocytes. Fibrosis progresses to irregular cirrhosis and leads to death from liver failure within the first decade and rarely after adolescence. A locus for PFIC1 was mapped to 18q21–q22 in the original Byler pedigree (11). This locus is the same as for benign recurrent intrahepatic cholestasis and was first identified by homozygosity mapping in patients with benign recurrent intrahepatic cholestasis (12). These findings led to the suggestion that the two diseases were caused by mutations in the same gene, despite their different phenotypes and prognoses (14). In this respect, it is interesting to note that while it is much more severe than benign recurrent intrahepatic cholestasis, Byler’s disease is also often initially characterized by bouts of jaundice which resolve spontaneously (6,18). PFIC1 is due to a defect of a single coding sequence, termed FIC1 (for Familial Intrahepatic Cholestasis 1), which has been identified by positional cloning within the 18q21–22 locus. This gene encodes a P-type ATPase which is potentially involved in the transport of amino-phospholipids (phosphatidylserine and phosphatidylethanolamine) from the outer to the inner leaflet of various cellular membranes (14). Several homozygous mutations of the FIC1 gene have been identified in children with PFIC1 and in patients with benign recurrent intrahepatic cholestasis, indicating that mutations in this gene are responsible for both disorders. In patients with PFIC1, five mutations have been identified,
TABLE 1
Main characteristics of progressive familial intrahepatic cholestasis

<table>
<thead>
<tr>
<th></th>
<th>PFIC1 (Byler’s disease)</th>
<th>PFIC2 (Byler syndrome)</th>
<th>PFIC3 (MDR3 deficiency)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transmission</td>
<td>Autosomal recessive</td>
<td>Autosomal recessive</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>Pruritus</td>
<td>severe</td>
<td>severe</td>
<td>moderate</td>
</tr>
<tr>
<td>Serum GGT activity</td>
<td>normal</td>
<td>normal</td>
<td>high</td>
</tr>
<tr>
<td>Ductular proliferation</td>
<td>absent</td>
<td>absent</td>
<td>present</td>
</tr>
<tr>
<td>Bile composition</td>
<td>Low primary bile acid concentration*</td>
<td>Low primary bile acid concentration</td>
<td>Low phospholipid concentration</td>
</tr>
<tr>
<td>Chromosomal locus</td>
<td>18q21–22</td>
<td>2q24</td>
<td>7q21</td>
</tr>
<tr>
<td>Gene/protein*</td>
<td>FIC1/P-type ATPase</td>
<td>SPGP*/SPGP*, (BSEP)</td>
<td>MDR3*/MDR3*</td>
</tr>
<tr>
<td>Hepatocyte location</td>
<td>Unknown, canalicular membrane?</td>
<td>Canalicular membrane</td>
<td>Canalicular membrane</td>
</tr>
<tr>
<td>Function defect</td>
<td>Unknown, ATP dependent amino-phospholipid transport?</td>
<td>ATP-dependent</td>
<td>ATP-dependent translocation of phosphatidylcholine into bile</td>
</tr>
</tbody>
</table>

*, These transporters belong to the ATP-binding cassette family. PFIC1, progressive familial intrahepatic cholestasis, Type 1; PFIC2, progressive familial intrahepatic cholestasis, Type 2; PFIC3, progressive familial intrahepatic cholestasis, Type 3; FIC1, gene involved in PFIC1; SPGP, sister of P-glycoprotein; BSEP, bile salt export pump; MDR3, multidrug-resistance-3 P-glycoprotein; GGT, gamma-glutamyltransferase; *, mainly affecting the concentration of chenodeoxycholic acid.

means that patients unrelated to the Byler family may also have FIC1 mutations. In patients with benign recurrent intrahepatic cholestasis, a missense mutation and a deletion have been found in less conserved domains of P-type ATPases. It is hypothesized that the mutations identified in patients with PFIC1 affect the protein function completely, while in patients with benign recurrent intrahepatic cholestasis it is partially affected (14). So far, there is no good explanation for the phenotypic difference between PFIC1 and benign recurrent intrahepatic cholestasis patients. The function of FIC1 is not yet known, but it is postulated that the defect of the FIC1 gene would impair the membrane distribution of aminophospholipids, thus indirectly disturbing the biliary secretion of bile acids (14). The FIC1 gene is expressed in various organs including the liver, pancreas, kidney and small intestine, but is more highly expressed in the small intestine than in the liver and is likely to play a part in the enterohepatic circulation of bile acids (14). This could explain the chronic watery diarrhea, present in a few children with PFIC1, which does not resolve after liver transplantation (14,18,20,21). A preliminary study performed on rat liver by in situ hybridization has shown that FIC1 mRNA is expressed in cholangiocytes and not in hepatocytes (22). Further studies such as a knock-out model and cell transfection studies should help understanding of the role of this P-type ATPase in bile formation and cholestasis. It will also be interesting to determine the factors which are responsible for attacks of jaundice in these patients.

**Progressive Familial Intrahepatic Cholestasis, Type 2 (PFIC2, Byler Syndrome)**

Patients with phenotypic findings resembling those of PFIC1 but unrelated to the original Byler family are
considered to have Byler syndrome and have been described all over the world (5–9,13,18,21,23,24). Patients with this type of PFIC (called PFIC2) also present with severe pruritus, normal serum gamma-glutamyltransferase activity and cholesterol level, high concentration of serum primary bile acids and low biliary primary bile acid concentration (5–9,18). Nevertheless, some slight phenotypic differences have been reported between PFIC1 and PFIC2. Initial presentation and evolution seem to be more severe with permanent jaundice from the onset and the rapid appearance of liver failure (5,6). Initial liver histology shows canalicular cholestasis and absence of a true ductular proliferation with periportal biliary metaplasia of hepatocytes as in PFIC1, but the liver architecture is more perturbed with lobular and portal fibrosis and inflammation, and a strong giant cell transformation is often present (5,6,18,19). Fibrosis progresses rapidly to irregular cirrhosis and leads to liver failure within the first years of life. These differences remain questionable and may only be confirmed when phenotype-genotype correlations become available. A gene locus for PFIC2 has been mapped to 2q24 in Middle Eastern patients in whom the PFIC1 locus was excluded (13). In parallel to this linkage analysis, the ATP-dependent canalicular bile acid transporter was cloned from rat liver (25). This transporter called the sister of P-glycoprotein (spgp) is exclusively expressed in the liver and represents the canalicular bile salt export pump (BSEP) of mammalian liver. A preliminary SPGP immunostaining study performed in the liver of children with a PFIC2 phenotype has shown that in some children no SPGP was detectable at the canalicular membrane, suggesting that a defect of SPGP underlies the PFIC2 phenotype (26). This has been confirmed by the recent cloning of the human SPGP which has been mapped to the 2q24 locus and the identification of 10 SPGP mutations in PFIC2 patients from several distinct populations (16). One or more of these mutations have been found in 25 PFIC2 families. In 16 families the affected individuals are homozygous, and one compound heterozygote has been identified. So far, in other families only one of the disease-causing mutations has been identified. All 10 mutations are predicted to be functionally significant, including mutations leading to premature truncation of the protein or missense changes in important domains of the protein. These findings are consistent with the decreased canalicular excretion of bile acids described in such patients (8,18). It is likely that impaired biliary bile acid secretion causes accumulation of bile acids inside the hepatocyte and ongoing severe hepatocellular damage. Further studies, such as a knock-out model, will confirm the role of SPGP in bile formation and cholestasis.

**Progressive Familial Intrahepatic Cholestasis, Type 3 (PFIC3, MDR3 Deficiency)**

Patients with this type of PFIC can be distinguished from those with the other types by a high serum gamma-glutamyltransferase activity and liver histology which shows portal fibrosis with ductular proliferation and inflammatory infiltrate in the early stages despite patency of intra- and extrahepatic bile ducts (9,15,27,28). Cytokeratin 19 immunostaining confirms the true ductular proliferation, and the evolution of liver histology is characterized by the appearance of a typical picture of biliary cirrhosis. By contrast, patients with PFIC3 usually present later in life, carry a higher risk of portal hypertension and gastrointestinal bleeding and develop liver failure at a later age. It is characterized by a mild and variable pruritus, moderately raised concentrations of serum primary bile acid and normal concentration of biliary primary bile acids (9,15,27,28). These differences suggest that a distinct etiological mechanism underlies this type of PFIC. Indeed, the pattern of non-suppurative cholangitis occurring in this disorder is very similar to the hepatic injury observed in mice with a homozygous disruption of the mdr2 gene (mdr2 −/− mice) (29). The murine mdr2 and the human MDR3 P-glycoproteins are phospholipid translocators involved in biliary phospholipid (phosphatidylcholine) excretion and are predominantly, if not exclusively, expressed in the canalicular membrane of the hepatocyte (30–34). It has been postulated that a genetic defect in the MDR3 gene, which is localized on 7q21, may be the cause of this type of PFIC which shares biochemical, histological and genetic features with mdr2 −/− mice (27). To investigate this possibility, the presence of phospholipids in bile and mRNA encoding the MDR3 P-glycoprotein have been tested in the liver of children with PFIC and high serum gamma-glutamyltransferase activity. An abnormal expression of the MDR3 gene (low amount of liver mRNA) and a low biliary phospholipid concentration have been found in such patients compared to those with other cholestatic disorders (27). In a subsequent study, MDR3 cDNA analysis, genomic DNA analysis and immunohistochemistry with a specific polyclonal antibody against MDR3 P-glycoprotein performed in two patients with this type of PFIC confirmed a MDR3 gene defect (15). Immunohistochemistry revealed the lack of canalicular staining for MDR3 P-glycoprotein in the liver tissue of both patients. In the first patient, a homozygous 7-bp deletion starting at amino acid 132 results in a frame-shift and stop codon
29 amino acids downstream. The homozygous and heterozygous status of genomic DNA of the patient and both parents was confirmed by PCR-SSCP analysis. In the second patient, a single-bp substitution at amino acid 957 results in a stop codon. This mutation deletes a TaqI restriction site which has been used to confirm the homozygous and heterozygous status of genomic DNA in the patient and both parents, respectively. In both patients truncated MDR3 P-glycoproteins will lack at least one ABC motif and if synthesized will therefore probably not be active. Additional nonsense mutations and missense mutations associated with low biliary phospholipid levels have been identified in similar patients and affected siblings (35). As in the murine model, the liver pathology may be due to a toxic effect of bile acids on bile canaliculi and the biliary epithelium in the absence of biliary phospholipids (29,33,36). Indeed, biliary phospholipids normally protect ductular epithelial cells from the toxicity of bile acids by forming mixed micelles. Interestingly, it has been found, within the families of two affected children, each child having a distinct nonsense homozygous MDR3 mutation, that heterozygous women had experienced typical recurrent episodes of intrahepatic cholestasis of pregnancy (15,37). These familial observations provide arguments for a genetic basis of intrahepatic cholestasis of pregnancy (15,37). Such events could favor the transient decompensation of the heterozygous state for a MDR3 gene defect during pregnancy, leading to intrahepatic cholestasis of pregnancy (37). As for PFIC3, cholestasis would result from the toxicity of bile in which detergent bile salts are not inactivated by phospholipids. While heterozygous mdr2 (+/−) mice, with a maximal phospholipid secretion of 60% of controls do not develop liver disease, the appearance of liver injury in a heterozygous patient could be expected because in humans the bile salt pool is much more hydrophobic than in mice (15,29,33,36). This may justify searching for an MDR3 gene mutation in intrahepatic cholestasis of pregnancy, particularly if serum gamma-glutamyltransferase activity is high (37). These data also raise the issue of whether a woman heterozygous for an FIC1 or SPGP defect may be at risk for intrahepatic cholestasis of pregnancy. Indeed, in the original report on Byler’s disease, the mother of an affected child had experienced several episodes of intrahepatic cholestasis of pregnancy (1,2).

Conclusion
Major advances in the understanding of the molecular mechanisms of bile formation have been made in the past few years. The impact of these advances and of molecular genetics in pediatric hepatology has allowed the establishment of the molecular basis of PFIC and demonstrated that genetic defects in the FIC1, SPGP and MDR3 genes are responsible for distinct PFIC phenotypes. These findings have confirmed the autosomal recessive inheritance of the disease and should allow prenatal diagnosis in the future. Identification of mutations in these genes will allow genotype/phenotype correlations to be defined within the spectrum of PFIC. These correlations performed in patients previously treated with ursodeoxycholic acid or biliary diversion should allow those PFIC patients who could benefit from these therapies to be precisely identified (38–40). In the future, other therapies such as cell and gene therapies might be considered and could also represent an alternative to liver transplantation (41,42). In addition, it remains to be clarified whether defects in the FIC1, SPGP and MDR3 genes are responsible for all types of PFIC, or if other genes, still to be discovered and potentially involved in bile formation or its regulation, may cause PFIC.

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References
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