The molecular genetics of familial intrahepatic cholestasis

There is a growing list of genetic diseases caused by defects of one of the members of the ATP binding cassette (ABC) transporter superfamily. ABC transporters mediate the energy dependent transport of peptides, steroid hormones, and drugs and their metabolites across membranes, not only in mammals but also in fish, bacteria, worms, and even plants. ABC transporters are important in almost every human cell or organ and therefore the spectrum of diseases caused by defects of these proteins is diverse and includes: liver diseases (progressive familial intrahepatic cholestasis, cystic fibrosis, Zellweger syndrome, adrenoleukodystrophy, and Dubin-Johnson syndrome); eye disorders (Stargardt disease, autosomal recessive retinitis pigmentosa, and cone-rod dystrophy); disorders of cholesterol metabolism (familial HDL deficiency and Tangier disease); and diseases of carbohydrate metabolism (familial persistent hyperinsulinaemic hypoglycaemia of infancy).

Progressive familial intrahepatic cholestasis type 1

Progressive familial intrahepatic cholestasis (PFIC) belongs to a group of autosomal recessive diseases characterised by cholestasis starting in infancy (table 1). PFIC type 1 or Byler disease often begins with recurrent episodes of intrahepatic cholestasis progressing to permanent cholestasis with fibrosis, cirrhosis, and liver failure necessitating liver transplantation in the first decade of life. Children with PFIC are small for their age and, in addition to cholestasis and pruritus, they sometimes have diarrhoea, pancreatitis, and hearing loss. The larger bile ducts are anatomically normal and histologically the liver shows a picture of bland canalicular cholestasis without much bile duct proliferation, inflammation, fibrosis, or cirrhosis. On electron microscopy there is a paucity of canalicular microvilli and a thickened pericanalicular network of microfilaments with coarse granular bile called “Byler bile” in the canaliculi. Characteristically serum gamma-glutamyltransferase (gamma-GT) activity is not increased or only slightly elevated while parameters of cholestasis such as alkaline phosphatase and serum primary bile acids in particular chenodeoxycholic acid) are greatly increased. Serum cholesterol levels are usually normal.

Many patients belong to the so-called Byler kindred: descendants of Jacob and Nancy Byler who emigrated in the late 18th century from Germany to the United States to become the founders of a large Amish kindred. Many patients outside the United States are unrelated to the Amish and the PFIC syndrome has been described in families in the Netherlands, Sweden, Greenland, and an Arab population. In the Amish and in some of the non-Amish families, the genetic defect was mapped to the FIC1 locus on chromosome 18q21-q22. This FIC1 locus was further characterised by detailed homozygosity mapping and gene scanning studies to a region encoding a member of a recently defined subfamily of P type ATPases (fig 1).

P type ATPases are not ABC transporters; they belong to a large family mainly encoding ion transport pumps such as Na’K’ ATPase, Ca++ ATPase, and the copper transporting Wilson protein ATP7B. The function of FIC1 appears to mediate the transport of aminophospholipids (that is phosphatidylserine) from the outer to the inner leaflet of plasma membranes. However, the debate about this protein continues. In humans, FIC1 is highly expressed in the pancreas, small intestine, urinary bladder, stomach, and prostate. This may explain the increased frequency of diarrhoea and pancreatitis in these patients but the relation with cholestasis, the hallmark of the disease, is not immediately apparent. For example, in the liver the protein is not highly expressed and is located in cholangiocytes, not in hepatocytes (see Mutero and colleagues). Therefore, the relation between the FIC1 locus and cholestasis is unclear.

Recurrent familial intrahepatic cholestasis

Recurrent familial intrahepatic cholestasis is a term recently coined by Tygstrup and colleagues. This disease is also known as benign recurrent intrahepatic cholestasis (BRIC) or Summerskill syndrome and was described by Summerskill and Walshe in 1959. Despite recurrent attacks of cholestasis there is no progression to chronic liver disease. During the attacks patients are severely jaundiced and have pruritus, steatorrhoea, and weight loss. As in PFIC 1, serum gamma-GT is not elevated. Some patients also have renal stones, pancreatitis, and diabetes. Tygstrup and colleagues proposed dropping the adjective “benign” from the name of this disease because sometimes the cholestatic episodes interfere so much with the social life of these patients that transplantation is warranted.

The gene involved in recurrent familial intrahepatic cholestasis has been mapped to the FIC1 locus. This suggests that recurrent and progressive familial intrahepatic cholestasis type I are genetically and perhaps also pathophysio logically related.

Abbreviations used in this paper: PFIC, progressive familial intrahepatic cholestasis; BRIC, benign recurrent intrahepatic cholestasis; gamma-GT, gamma-glutamyltransferase; BSEP, bile salt export pump; cMOAT, canalicular multispecific organic anion transporter; MRP2, multidrug resistance protein 2; POY3, P-glycoprotein 3; ABC, ATP binding cassette.
Not mentioned in the table is Aagenaes syndrome (intrahepatic cholestasis with lymphoedema) since the gene defect underlying this disease is unknown.

PFIC, progressive familial intrahepatic cholestasis; BRIC, benign recurrent intrahepatic cholestasis; ICP, intrahepatic cholestasis of pregnancy; PGY, P-glycoprotein; gene. This gene encodes the canalicular bile salts export transporter, including that of bilirubin conjugates.

**Table 1 Genetic forms of intrahepatic cholestasis or hyperbilirubinaemia**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Chromosome</th>
<th>Gene</th>
<th>Defect</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFIC type 1</td>
<td>18q21</td>
<td>FITC1</td>
<td>Pathogenetic mechanism unknown</td>
<td>First recurrent, later permanent and progressive cholestasis, no bile duct proliferation, normal gamma-GT, extrahepatic manifestations in some patients</td>
</tr>
<tr>
<td>BRIC</td>
<td>18q21</td>
<td>FITC1</td>
<td>Unknown but most likely a regulatory defect of bile salt secretion</td>
<td>Recurrent attacks of severe cholestasis, pruritus, jaundice, steatorrhoea, and weight loss. Normal liver function in intervals between attacks</td>
</tr>
<tr>
<td>PFIC type 2</td>
<td>2q24</td>
<td>BSEP</td>
<td>Deficient canalicular bile salt transport</td>
<td>Progressive cholestasis, no bile duct proliferation, giant cell transformation, lobular and portal fibrosis, normal gamma-GT</td>
</tr>
<tr>
<td>PFIC type 3</td>
<td>7q21</td>
<td>PGY3</td>
<td>Deficient canalicular phosphatidylincholine transport</td>
<td>Cholestasis, jaundice less prominent, extensive bile duct proliferation and periporal fibrosis, elevated gamma-GT</td>
</tr>
<tr>
<td>ICP</td>
<td>e.g. 7q21</td>
<td>e.g. PGY3</td>
<td>May be associated with e.g. PFIC type 3 but is also associated with other PFIC types</td>
<td>Cholestasis in third trimester of pregnancy, therapeutic effect of ursodeoxycholic acid, associated with increased fetal loss and prematurity</td>
</tr>
<tr>
<td>Bile acid synthesis defects</td>
<td>e.g. 8q2.3</td>
<td>e.g. CYP/BI</td>
<td>Bile acid synthesis enzyme defects with accumulation of toxic intermediates and deficiency of normal bile acids</td>
<td>Cholestasis since birth, failure to thrive, low to normal gamma-GT</td>
</tr>
<tr>
<td>Dubin-Johnson syndrome</td>
<td>10q24</td>
<td>MRP2/MOAT</td>
<td>Deficient canalicular organic anion transport, including that of bilirubin conjugates</td>
<td>Conjugated hyperbilirubinaemia, increased urinary coproporphyrin isomer I, hepatic lysosomal pigment, normal life span</td>
</tr>
</tbody>
</table>

**Progressive familial intrahepatic cholestasis type 2**

Genetic studies revealed that the FITC1 locus was not involved in all patients with a PFIC type 1 phenotype and low serum gamma-GT. In a number of patients the disease was mapped to a locus on chromosome 2q24 which later proved to be the BSEP (bile salt export pump) gene. This gene encodes the canalicular bile salts export pump, a P-glycoprotein belonging to class B of the ABC transporter superfamily (for classification and overview of all known members of the ABC transporter superfamily see http://www.med.rug.nl/mlh2/humanabc.htm). This protein was originally called “sister of P-glycoprotein”. Similar proteins in pigs, rats, and mice have a great degree of homology with human BSEP and antibodies directed against sequences at the carboxy terminus display cross species reactivity. This enabled localisation studies and it became clear that this protein is liver specific and is located in the canalicular domain of the plasma membrane of the hepatocyte. In a recent collaborative study we showed that in patients with PFIC type 2, canalicular staining with specific BSEP antibodies was negative and that all of these patients carry mutations in the BSEP gene (fig 2). As in PFIC type 1, serum gamma-GT activity in these patients is not elevated and bile duct proliferation is absent. However, there are also some differences from PFIC type 1: in PFIC 2 the disease frequently starts as non-specific giant cell hepatitis which is indistinguishable from idiopathic neonatal giant cell hepatitis; patients are usually permanently jaundiced and the disease rapidly progresses to persistent and progressive cholestasis requiring liver transplantation. Histologically the liver shows more inflammatory activity, giant cell transformation, and lobular and portal fibrosis than in PFIC. The bile of PFIC type 2 patients is amorphous or filamentous on electron microscopy. Extrahepatic manifestations are uncommon. PFIC type 2 patients do not respond to ursodeoxycholic acid therapy, in fact administration of ursodeoxycholic acid to some of these patients caused very high serum bile acid levels (>1 mmol/l) without any increase in biliary bile acid secretion. This is additional proof that the primary defect in these patients is a defective canalicular bile acid transport pump (fig 3).

Bile acids are not completely absent in the bile of these patients. Multidrug resistance protein 2 (MRP2 or

**Figure 1** Putative structure of FITC1. The FITC1 gene has been demonstrated to be mutated in patients with progressive familial intrahepatic cholestasis (PFIC) type 1 and benign recurrent intrahepatic cholestasis (BRIC). It encodes a membrane protein with 10 putative transmembrane domains that exhibits homology with proteins with presumed aminophospholipid translocase activity. The green boxes represent P type ATPase signature domains; the red symbols mark mutations.

**Figure 2** Putative structure of BSEP. The BSEP (bile salt export pump) gene has been demonstrated to be mutated in patients with progressive familial intrahepatic cholestasis (PFIC) type 2. It encodes a membrane protein with 12 putative transmembrane domains that functions as a major bile salt export pump. The white boxes represent the Walker A and B motifs and the “ABC” signature; the red symbols mark mutations (modified after Strautnieks and colleagues).
canalicular multispecific organic anion transporter (cMOAT)), the canalicular transporter of bilirubin and other organic anions, also transports glucuronidated or sulphated bile acids. This transporter may act as an escape pathway under conditions of cholestasis. This may also explain why these patients are jaundiced despite an intact bilirubin transporter: bilirubin transport may be competitively inhibited by bile acid conjugates.

Bile acid synthesis defects
Defects of bile acid synthesis may resemble PFIC type 2. Clayton et al and Setchell et al described a defect in \( \Delta^\alpha-\Delta^\beta \)-27-hydroxysteroid oxidoreductase as a cause of giant cell hepatitis, a condition also frequently seen at the onset of PFIC type 2. Deficiency of \( \Delta^\Delta-\Delta^\beta \)-hydroxy C27steroid dehydrogenase/isomerase and mutations of the oxysterol 7alpha-hydroxylase gene (CYP7B1) may also be causes of neonatal hepatitis and cholestasis. In these diseases toxic intermediates are formed which cause cholestasis by interaction with the hepatic bile acid transporter. Bile acid synthesis defects are called PFIC type 4 by some authors.

Progressive familial intrahepatic cholestasis type 3
The third PFIC subtype, PFIC type 3, is quite different from the other PFIC subtypes. Serum gamma-GT activity is usually markedly elevated in these patients and the liver histology shows extensive bile duct proliferation, and portal and periporal fibrosis. Phenotypically PFIC type 3 resembles the mdr 2 \(-/-\) mice who have homozygous disruption of mdr2, a canalicular phospholipid translocator. In humans, mdr 2 is called MDR3 or P-glycoprotein 3 (PGY3) and the gene encoding this protein is mutated in this disease (fig 4). MDR3/mdr2 acts as a flippase, moving phospholipids from the inner leaflet of the canalicular membrane to the outer leaflet which faces the canalicular lumen. In common with BSEP, MDR3/mdr2 is a P-glycoprotein belonging to class B of the ABC transporter superfamily.

Phosphatidylcholine, the predominant phospholipid in bile, is washed down from the canalicular membrane into the bile by bile acids. Thus without bile acids, as in PFIC type 2, bile is devoid of phospholipids. Without MDR3, as in PFIC type 3, bile acid transport proceeds unimpaired. This has major consequences because in normal bile the inherent toxicity of bile acids is quenched by phosphatidylcholine. In the bile of PFIC 3 patients (and mdr 2 \(-/-\) mice) bile acid monomers are highly toxic to cholangiocytes and hepatocytes. In humans this is even more extreme than in mdr 2 \(-/-\) mice as the monohydroxy bile acids of humans are more toxic than the muricholic acids of mouse bile.

In patients with PFIC type 3, symptoms present somewhat later in life than in PFIC types 1 and 2 and liver failure also occurs at a later age. Jaundice may be less apparent. Some of these patients respond to ursodeoxycholic acid therapy but liver transplantation is eventually often necessary.
Mutations of the MDR3 gene on chromosome 7q21 is the underlying cause of the disease. Although PFIC 3 is discussed as a cholestatic disease, in a strictly physiological sense there is no cholestasis as bile flow is not impaired. The bile in these patients is toxic and this causes the cholestatic type of liver damage described above.

Intrahepatic cholestasis of pregnancy

Jacquemin et al reported a high incidence of intrahepatic cholestasis of pregnancy in one of their families with PFIC type 3. This suggests that in subjects carrying one mutated PGY3 gene, cholestasis may occur during pregnancy. Intrahepatic cholestasis of pregnancy has also been described in families with other PFIC subtypes.

Dubin-Johnson syndrome

Dubin-Johnson syndrome is described here, not because it is a mutation of an ABC transporter in the liver, but because is is a cause of a cholestatic disease, in a strictly physiological sense there is no cholestasis as bile flow is not impaired. The bile in these patients is toxic and this causes the cholestatic type of liver damage described above.

Conclusion

What do unlikely neighbours such as Stargardt disease, progressive familial intrahepatic cholestasis, and familial persistent hyperinsulinaemic hypoglycaemia of infancy have in common? They are all diseases caused by mutations of one of the members of the ABC transporter superfamily. Do these novel views provide any benefit for patients with these diseases? For most of the PFIC patients liver transplantation will remain necessary for some time to come but eventually gene therapy or transplantation of genetically corrected autologous hepatocytes may become a reality. Analysis of the aetiology of this heterogeneous group of Byler-like diseases has been a first and necessary step.
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