

Gut

Leading article

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.¹ 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,² cystic fibrosis,³ Zellweger syndrome,⁴ adrenoleukodystrophy,⁵ and Dubin-Johnson syndrome⁶); eye disorders (Stargardt disease,⁷ autosomal recessive retinitis pigmentosa,⁸ and cone-rod dystrophy⁹); disorders of cholesterol metabolism (familial HDL deficiency¹⁰ and Tangier disease¹⁰); and diseases of carbohydrate metabolism (familial persistent hyperinsulinaemic hypoglycaemia of infancy¹¹).

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.^{2 12-14} Children with PFIC are small for their age and, in addition to cholestasis and pruritus, they sometimes have diarrhoea, pancreatitis, and hearing loss.¹⁵ 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.^{14 16} 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.^{12-14 18 19} 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, Ca²⁺ ATPase, and the copper transporting Wilson protein ATP7B. The function of *FIC1* is uncertain. Its homologue, the bovine P type ATPase II, appears to mediate the transport of aminophospholipids (that is phosphatidylserine) from the outer to the inner leaflet of plasma membranes.^{21 22} However, the debate about this protein continues.²³ 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 Muter and colleagues²⁴). 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.²⁵ This disease is also known as benign recurrent intrahepatic cholestasis (BRIC) or Summerskill syndrome and was described by Summerskill and Walshe in 1959.²⁶ 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.²⁵ 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.^{20 27 28} This suggests that recurrent and progressive familial intrahepatic cholestasis type I are genetically and perhaps also pathophysiologically 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; PGY3, P-glycoprotein 3; ABC, ATP binding cassette.

Table 1 Genetic forms of intrahepatic cholestasis or hyperbilirubinaemia

Disease	Chromosome	Gene	Defect	Phenotype
PFIC type 1	18q21	<i>FIC1</i> , P type ATPase with homology to a putative aminophospholipid translocator	Pathogenetic mechanism unknown	First recurrent, later permanent and progressive cholestasis, no bile duct proliferation, normal gamma-GT, extrahepatic manifestations in some patients
BRIC	18q21	<i>FIC1</i>	Unknown but most likely a regulatory defect of bile salt secretion	Recurrent attacks of severe cholestasis, pruritus, jaundice, steatorrhoea, and weight loss. Normal liver function in intervals between the attacks
PFIC type 2	2q24	<i>BSEP</i> , bile salt export pump	Deficient canalicular bile salt transport	Progressive cholestasis, no bile duct proliferation, giant cell transformation, lobular and portal fibrosis, normal gamma-GT
PFIC type 3	7q21	<i>PGY3</i>	Deficient canalicular phosphatidylcholine transport	Cholestasis, jaundice less prominent, extensive bile duct proliferation and periportal fibrosis, elevated gamma-GT
ICP	e.g. 7q21 heterozygosity	e.g. <i>PGY3</i>	May be associated with e.g. PFIC type 3 but is also associated with other PFIC types	Cholestasis in third trimester of pregnancy, therapeutic effect of ursodeoxycholic acid, associated with increased fetal loss and prematurity
Bile acid synthesis defects	e.g. 8q2.3	e.g. <i>CYP7B1</i>	Bile acid synthesis enzyme defects with accumulation of toxic intermediates and deficiency of normal bile acids	Cholestasis since birth, failure to thrive, low to normal gamma-GT
Dubin-Johnson syndrome	10q24	<i>MRP2/cMOAT</i> , canalicular multispecific organic anion transporter	Deficient canalicular organic anion transport, including that of bilirubin conjugates	Conjugated hyperbilirubinaemia, increased urinary coproporphyrin isomer I, hepatic lysosomal pigment, normal life span

PFIC, progressive familial intrahepatic cholestasis; BRIC, benign recurrent intrahepatic cholestasis; ICP, intrahepatic cholestasis of pregnancy; PGY, P-glycoprotein; CYP, cytochrome P-450; gamma-GT, gamma-glutamyltransferase.

Not mentioned in the table is Aagaenaes syndrome (intrahepatic cholestasis with lymphoedema)¹⁷ since the gene defect underlying this disease is unknown.

Progressive familial intrahepatic cholestasis type 2

Genetic studies revealed that the *FIC1* locus was not involved in all patients with a PFIC type 1 phenotype and low serum gamma-GT.^{13, 14} 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.²⁹⁻³¹ 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/mdl/humanabc.htm>). This protein was originally called "sister of P-glycoprotein".^{32, 33} 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* gene³⁴ (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.^{2, 14, 35} 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.³⁴ 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

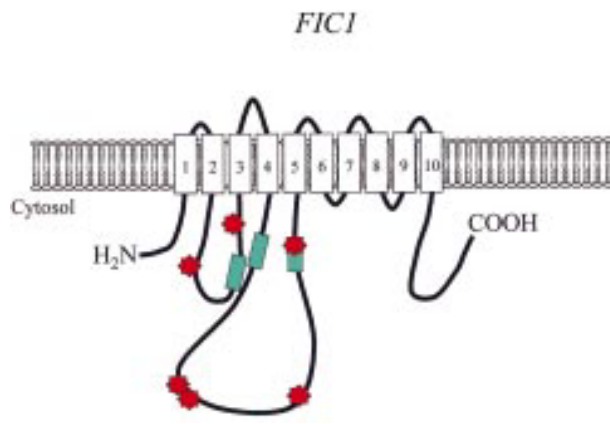


Figure 1 Putative structure of *FIC1*. The *FIC1* gene has been demonstrated to be mutated in patients with progressive familial intrahepatic cholestasis (PFIC) type 1 and benign recurrent intrahepatic cholestasis (BRIC).²⁰ It encodes a membrane protein with 10 putative transmembrane domains that exhibits homology with proteins with presumed aminophospholipid translocase activity. The green boxes represent P type ATPase signature domains; the red symbols mark mutations.

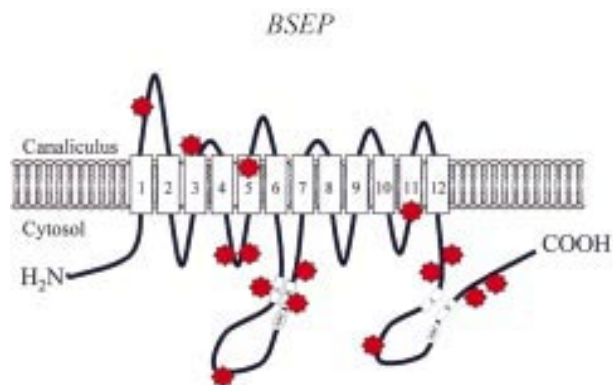


Figure 2 Putative structure of *BSEP*. The *BSEP* (bile salt export pump) gene has been demonstrated to be mutated in patients with progressive familial intrahepatic cholestasis (PFIC) type 2.^{31, 32} It encodes a membrane protein with 12 putative transmembrane domains that functions as a major bile salt export pump.^{32, 33} The white boxes represent the Walker A and B motifs and the "ABC" signature; the red symbols mark mutations (modified after Strautnieks and colleagues³¹).

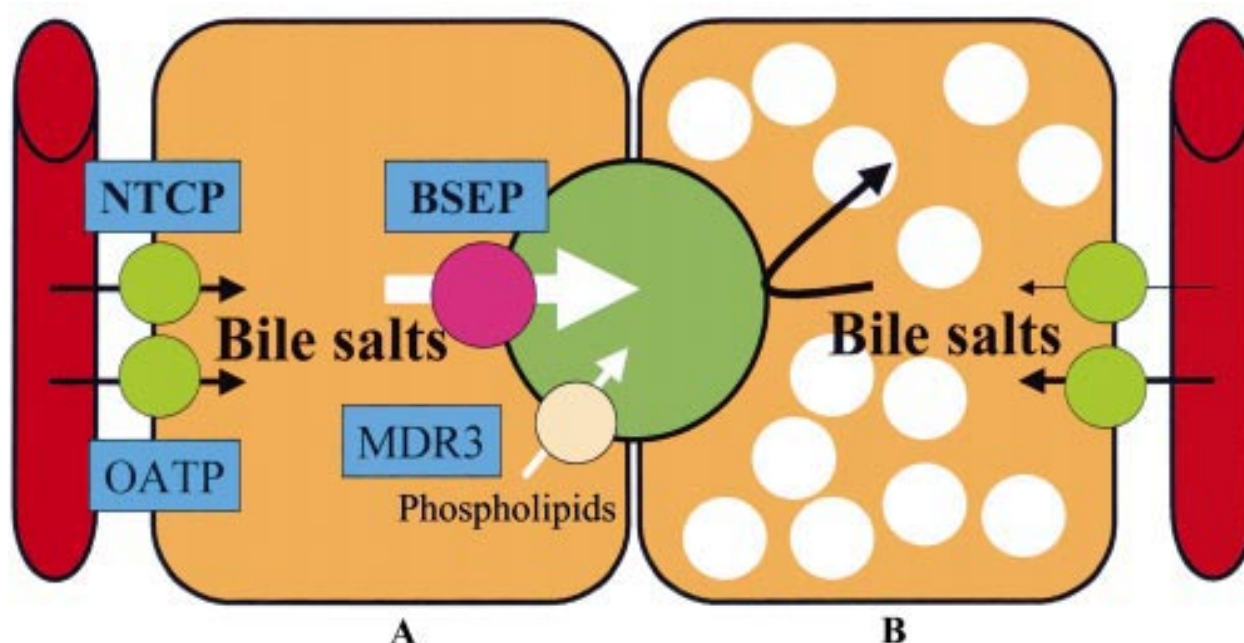


Figure 3 Bile salt transport. (A) Bile salts are taken up from the blood into the hepatocyte via carrier proteins in the basolateral membrane. These are NTCP or “sodium taurocholate cotransporting protein” and OATP or “organic anion transporting protein”. At the canalicular membrane bile salts are transported into the bile canaliculus by the ATP dependent “bile salt export pump” (BSEP). Hepatocanalicular transport of phospholipids, mainly phosphatidylcholine, is mediated by the P-glycoprotein MDR3. (B) In patients with progressive familial intrahepatic cholestasis type 2, BSEP is not expressed.

canalicular multispecific organic anion transporter (cMOAT)), the canalicular transporter of bilirubin and other organic anions,^{35–37} also transports glucuronidated or sulphated bile acids.³⁸ 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 3β - $\Delta 5$ -C27-hydroxysteroid oxidoreductase as a cause of giant cell hepatitis,³⁹ a condition also frequently seen at the onset of PFIC type 2. Deficiency of $\Delta 4$ -3-oxosteroid-5 β reductase and 3β -hydroxy C27steroid dehydrogenase/isomerase and mutations of the oxysterol 7 α -hydroxylase gene (CYP7B1) may also be causes of neonatal hepatitis and cholestasis.^{40–42} In these diseases toxic intermediates are formed which cause cholestasis by interaction with the hepatic bile acid transporter.⁴³ 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 periportal fibrosis.⁴⁴ Phenotypically PFIC type 3 resembles the *mdr 2* $-/-$ mice who have homozygous disruption of *mdr 2*, a canalicular phospholipid translocator.⁴⁶ 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).^{44–47} MDR3/*mdr 2* 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/*mdr 2* is a P-glycoprotein belonging to class B of the ABC transporter superfamily.

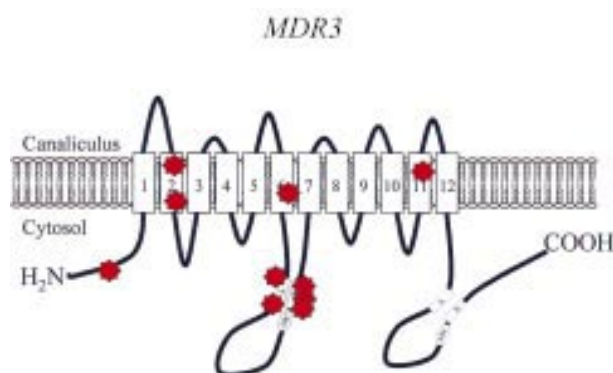


Figure 4 Putative structure of MDR3. The MDR3 gene has been demonstrated to be mutated in patients with progressive familial intrahepatic cholestasis (PFIC) type 3.^{44–47} It encodes a membrane protein with 12 putative transmembrane domains that functions as a phosphatidylcholine translocator. The white boxes represent the Walker A and B motifs and the “ABC” signature; the red symbols mark mutations.

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 therapy⁴⁸ but liver transplantation is eventually often necessary.

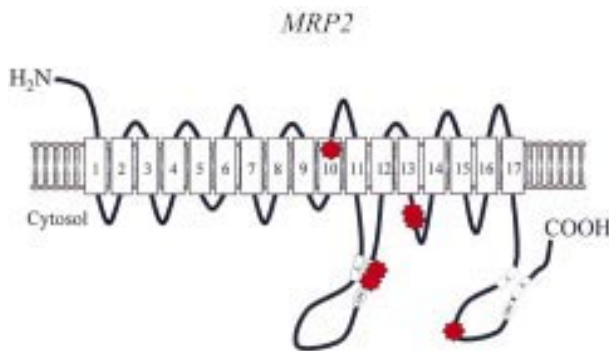


Figure 5 Putative structure of MRP2. The MRP2 gene has been demonstrated to be mutated in patients with Dubin-Johnson syndrome^{6, 52-54}. It encodes a membrane protein with 17 putative transmembrane domains that functions as a major export pump for anionic conjugates. The white boxes represent the Walker A and B motifs and the "ABC" signature; the red symbols mark mutations.

Mutations of the *MDR3* gene on chromosome 7q21 is the underlying cause of the disease.^{44, 45, 47} Although PFIC 3 is discussed as a cholestatic disease, in a strictly physiological sense there is no cholestasis as bile flow is not impaired.⁴⁶ 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 3.⁴⁷ 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.⁴⁹⁻⁵¹

Dubin-Johnson syndrome

Dubin-Johnson syndrome is described here, not because it is an important cholestatic disease, but because it is caused by a mutation of an ABC transporter in the liver^{6, 52-54} (fig 5). Dubin-Johnson syndrome is characterised by conjugated hyperbilirubinaemia without other serum enzyme abnormalities. Patients with Dubin-Johnson syndrome have a normal life span. A black or brownish lysosomal pigment in the hepatocytes is a characteristic histological feature and urinary coproporphyrin isomer I excretion is elevated.

The so-called TR- rat is an animal model for this disease. These animals have decreased hepatobiliary secretion of organic anions because of a mutation of the *cmoat/mrp2* gene.⁵⁵⁻⁵⁸ cMOAT, also called MRP2, is a member of the ABC transporter subfamily C of multidrug resistance associated proteins which includes CFTR, the cystic fibrosis transmembrane regulator, and SUR involved in familial persistent hyperinsulinaemic hypoglycaemia of infancy. Patients with the Dubin-Johnson syndrome are homozygous carriers of *cMOAT/MRP2* gene mutations.^{6, 52-54}

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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