

Cholestasis

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In contrast with urine formation, bile flow is not dependent on hydrostatic forces, but driven by osmotic pressure of solutes secreted across the apical membrane of hepatocytes and bile duct epithelial cells. This secretory process is mediated by a set of primary active transporters that use ATP hydrolysis to pump solutes against the concentration gradient. The most important solutes in bile are bile salts, lipids, electrolytes, and organic anions. The direct consequence of the osmotic mechanism of bile formation is that impaired function of these pumps leads to impaired bile flow—that is, cholestasis. The function of these pumps is highlighted by a number of inherited cholestatic diseases, which are caused by mutations in these genes. Identification of the molecular defect in these diseases was not only important for diagnostic reasons but also emphasised that impaired transporter function has pathological consequences. Indeed, it is now becoming clear that impaired or downregulated transporter function is also involved in the pathogenesis of acquired cholestatic syndromes.

The smallest functional unit of the liver is the lobule in which hepatocytes are arranged in plates along which blood flows from portal to central veins. Within these plates the small apical domains of adjacent hepatocytes form a tubular lumen, the canaliculus, which is the site of primary bile formation. From the canalicular network bile flows to the small ductules and subsequently to the larger ducts (table 1).

Bile formation is an osmotic process, which means that the generation of water flow is preceded by the active secretion of solutes, followed by the osmotic attraction of water. Solute secretion at the canalicular level is a process that is mediated by primary active transport-

ers. The main constituents of the primary biliary fluid are bile salts; hence flow mainly depends on the extent of bile salt secretion. Indeed, classic experiments have demonstrated in several species^{1,2} that there is a more or less linear relation between bile salt output and bile flow. Extrapolation of the flow to very low rates of bile salt secretion suggests that a so called bile salt independent fraction of bile flow also exists and more recent studies indeed indicated that canalicular glutathione secretion^{3,4} and ductular bicarbonate secretion⁵ also contribute to overall hepatic bile flow. The main determinant of overall bile flow is the volume of water generated at the canalicular level.

THE ENTEROHEPATIC CYCLE OF BILE SALTS

Hepatic uptake of bile salts is very efficient and is mainly mediated primarily by the Na⁺-taurocholate cotransporting peptide, NTCP⁶ (official gene code *SLC10A1*). As this transport is driven by the sodium gradient, it is capable of concentrating bile salts in the hepatocyte. This protein is exclusively expressed in the liver and localised in the basolateral membrane of the hepatocyte. NTCP has affinity for both conjugated and unconjugated bile salts.^{7–9} It was thought for a long time that intracellular transport of bile salts might involve vesicular transport, but there is no definitive proof for this and the current paradigm is that bulk bile salt transport only involves binding to cytosolic bile salt binding proteins. The most important of these proteins is 3 α OH-steroid dehydrogenase, which is present in extremely high concentration in the hepatocyte

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Abbreviations: NTCP, Na⁺-taurocholate cotransporting peptide; BSEP, bile salt export pump; ASBT, apical bile salt transporter; PC, phosphatidylcholine; GGT, γ -glutamyltransferase; BRIC, benign recurrent intrahepatic cholestasis; PRIC, progressive familial intrahepatic cholestasis type 2; ICP, intrahepatic cholestasis of pregnancy

Table 1 Transporters involved in primary bile formation

Transporter trivial name(s)	Gene code	Substrate(s)	Defective in
FIC1	<i>ATP8B1</i>	?	PFIC type 1
BSEP (sPgp)	<i>ABCB11</i>	bile salts	PFIC type 2
MDR3 Pgp (in mouse: Mdr2 Pgp)	<i>ABCB4</i>	phosphatidylcholine	PFIC type 3
MDR1 Pgp (in mouse: Mdr1a and 1b Pgp)	<i>ABCB1</i>	amphipathic drugs (neutral and cationic)	?
MRP2 (cMOAT)	<i>ABCC2</i>	amphipathic drugs (anionic and neutral)	Dubin Johnson syndrome
BCRP (MXR, ABCP)	<i>ABCG2</i>	amphipathic drugs	?
ABCG5	<i>ABCG5</i>	(phytosterols)	Sitosterolaemia
ABCG8	<i>ABCG8</i>	no direct evidence yet	

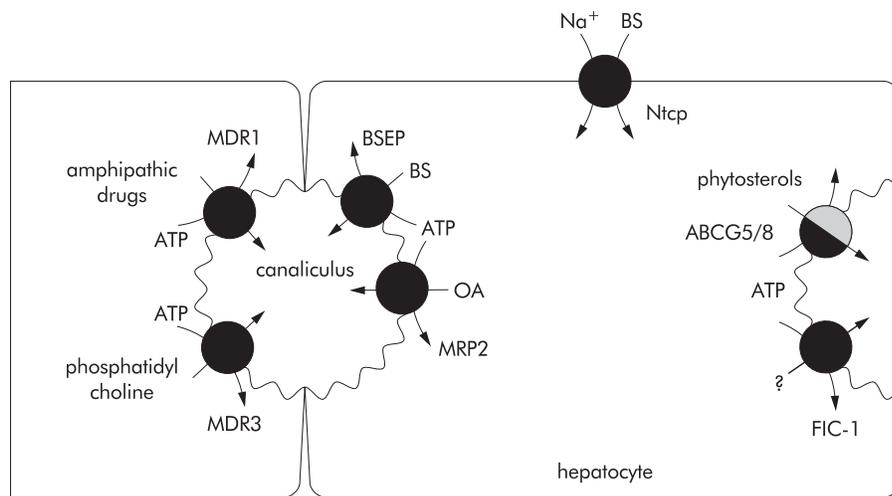


Figure 1 Transporters involved in canalicular bile formation. In the left canalicular membrane the ABC transporters are indicated, of which the function has been established. In the right canalicular membrane the heterodimer of ABCG5 and ABCG8 has been drawn, but this is speculative because the presence of these two half transporters in the canalicular membrane has not been demonstrated yet. It has also not yet been proved that phytosterols are transported by this transporter pair.

cytosol.^{10–11} This binding seems important not only in view of the detergent properties of bile salts at higher concentrations but also because of their potent ability to induce apoptosis in hepatocytes at low concentrations.^{12–13}

Bile salts are transported across the canalicular membrane in a primary active ATP dependent fashion,^{14–15} which is mediated by the bile salt export pump (BSEP, gene code *ABCB11*).^{16–19} BSEP is a genuine ATP binding cassette (ABC) transporter according to the classic paradigm of 12 transmembrane spanning domains and ATP binding folds. Different members of the family of ABC transporters are expressed in many tissues of the body and many of them are involved in active outward transport of molecules across the plasma membrane.

After reaching the intestine, bile salts are efficiently resorbed in the terminal ileum in a sodium dependent mechanism by the apical bile salt transporter (designated ASBT and formerly IBAT, official gene code: *SLC10A2*).^{20–22} When transfected in COS cells, the human ASBT²² recognises both primary and secondary bile salts in their unconjugated as well as their taurine conjugated form. Within the enterocyte bile salts are bound to the ileal bile salt binding protein. It was demonstrated recently that the expression of this protein is strongly regulated by the prevailing bile salt concentrations, suggesting that it may play a part in the regulation of the enterohepatic cycle of bile salts. This regulation is driven by the nuclear receptor pair FXR/RXR.²³ FXR recognises dihydroxy bile salts like deoxycholate and chenodeoxycholate.²⁴ It is now becoming clear that nuclear receptors regulate several steps in bile salt metabolism and transport.^{25–31}

How bile salts leave the enterocyte at the basolateral pole is currently unknown. MRP3 (official gene code *ABCC3*) was recently identified as yet another organic anion transporter.^{32–37} This protein is localised in the basolateral membrane of enterocytes and it has been shown to transport bile salts.³⁷ Whether MRP3 is the physiologically relevant transporter for release of bile salts into the blood remains to be determined. It is interesting to note that there is also low expression of MRP3 in the liver, which is strongly induced in different types of cholestasis. In contrast with the canalicular MRP2, MRP3 is localised in the basolateral membrane of hepatocytes.^{35–38} Thus, increased *MRP3* expression may represent an escape route for the excretion of accumulated organic anions to the blood that are normally excreted into bile, but in the absence of MRP2 cannot leave the hepatocyte.

The above described mechanisms ensure that bile salts undergo extensive enterohepatic circulation during which only small amounts (1%–3% per cycle) are lost. It must be emphasised that this description of the mechanism of entero-

hepatic bile salt cycling only holds for the main bile salts—that is, taurine or glycine conjugated cholate and chenodeoxycholate. Within the gut there is extensive deconjugation and dehydroxylation, which gives rise to many secondary bile salts and bile salt metabolites several of which have cholestatic properties. Depending on the species under study, these metabolites can be more or less efficiently rehydroxylated and conjugated to yield less toxic bile salts. An important example of this is lithocholate, the bacterial dehydroxylation product of chenodeoxycholate. The exact mechanisms of enterohepatic cycling of such metabolites has not sufficiently been studied. This will nevertheless be very important in the face of the potent cholestatic properties of some of these compounds.

BILIARY SECRETION OF LIPIDS

The second most important class of biliary constituents are the lipids. Biliary lipids mainly consists of phospholipid (almost exclusively phosphatidylcholine (PC)) and cholesterol. The comparatively high concentration of cholesterol in human bile (as compared with that of other species) represents the major risk factor to gallstone formation. Upon production of a knockout mouse for the *Mdr2* gene it was discovered that the encoded transporter is essential for biliary lipid secretion.³⁹ In the absence of *Mdr2* Pgp mice do not secrete phospholipid nor cholesterol. In different experimental systems it was subsequently demonstrated that the murine *Mdr2* Pgp as well as the human orthologue *MDR3* Pgp are PC translocators.^{40–42}

On the basis of available data, we have proposed a model⁴³ in which PC, after delivery to the inner leaflet of the canalicular plasma membrane, is translocated to the outer leaflet and subsequently resides in PC rich lipid domains that are laterally separated from the more rigid sphingolipid rich lipids of the outer canalicular membrane leaflet. This rigid lipid layer is necessary to prevent solubilisation of the canalicular membrane by high bile salt concentrations in the canaliculus (fig 1).

The mechanism of cholesterol secretion into bile is still largely unknown. As yet, no direct evidence exists for the involvement of a translocator protein which, in analogy with *Mdr2/MDR3* Pgp for phospholipids, would catalyse the translocation of cholesterol across the membrane. Controversy exists on the rate of spontaneous flip-flop of cholesterol across biological membranes. Cholesterol is not secreted into bile in the absence of phospholipid secretion; this is caused by the fact that simple bile salt micelles have very poor cholesterol solubilising capacity, especially those of the more hydrophilic bile salt species, such as muricholate (the main murine bile

salt) and ursodeoxycholate. This observation does, however, not exclude the possibility that cholesterol translocation needs a transporter protein. Indeed, recent evidence suggests that the two half transporters ABCG5 and ABCG8, which are expressed in liver and intestine, are involved in the elimination of plant sterols.^{44,45} Transport has thus far not been demonstrated directly, but this function can be inferred from accumulation of plant sterols in patients with sitosterolaemia, who have a mutation in either of the two genes encoding these proteins

BILIARY SECRETION OF BILIRUBIN, DRUGS, AND TOXINS

Thus far, three ABC transporters for amphipathic compounds have been identified in the canalicular membrane: MDR1 P-glycoprotein (gene code *ABCB1*), MRP2 (gene *ABCC2*) and BCRP (gene code *ABCG2*). In transfected cells and/or selected cell lines all three transporters confer multidrug resistance against a wide spectrum of cytotoxic agents. MDR1 P-glycoprotein pumps many neutral and positively charged organic compounds across the plasma membrane out of the cell. In view of the diversity of transported compounds, the definition of its substrate specificity has remained difficult. Clearly, molecular weight, bulkiness of the molecule, and charge of the molecule are important parameters.⁴⁶ The role of MDR1 Pgp in the protection against xenobiotics has been firmly established by the generation of knockout mice for both the *Mdr1a* and *Mdr1b* gene (in mice two *Mdr1* genes fulfill the function of the single *MDR1* gene in humans). This knockout animal is hypersensitive to a number of toxic compounds and important sites of expression are the blood-brain barrier, the intestinal epithelium, and the hepatocanalicular membrane.^{47,48} Thus far, no human disorders are known that are associated with a defect in the *MDR1* gene. It is of interest to note, however, that Panwala *et al*⁴⁹ detected a high incidence of colitis in *Mdr1a*^{-/-} mice. This chronic intestinal inflammation could be prevented by treatment with a mix of broad spectrum antibiotics, suggesting that intestinal bacteria and/or their products play a part in this phenomenon. Polymorphisms have been detected in the *MDR1* gene^{50,51} and these may be associated with alterations in the oral bioavailability of certain farmaca.⁵² The polymorphism C3435T was found to be associated with reduced MDR1 Pgp expression in the duodenum and this correlated with increased oral bioavailability of digoxin.⁵⁰ On the other hand no change in the bioavailability of cyclosporin A was observed.⁵³

MRP2 (gene code *ABCC2*) transports a wide spectrum of organic anions including conjugated bilirubin and other glucuronide conjugates as well as glutathione and sulphate conjugates. MRP2 also transports unconjugated organic anions, like the antibiotic ceftriaxone, a cephalosporin that is extremely efficiently excreted into bile. More recently, it has become clear that MRP2 can also accommodate uncharged amphipathic compounds together with a glutathione molecule in a co-transport mechanism. Experiments with plasma membrane vesicles containing MRP1 revealed that this transporter does not pump vincristine to any significant extent. However, addition of GSH strongly stimulated the translocation of vincristine.⁵⁴ Similar involvement of GSH was found for MRP2 mediated transport of the uncharged, food derived carcinogen PhIP.⁵⁵ This mechanism greatly increases the versatility of these transporters. In the intestine MRP2 may play a similar part as MDR1 Pgp in the defence against toxic compounds in the gut lumen.⁵⁵

Another member of this subfamily, MRP3, was already mentioned above. This transporter is also expressed in the basolateral membrane of cholangiocytes and portal hepatocytes. During cholestasis its expression is highly induced in

both cell types. It may function as a rescue mechanism for the cholestatic hepatocytes to prevent intracellular accumulation of bile salts and organic anions (see below).

More recently, BCRP (breast cancer related protein, also termed MXR or ABCP, gene code *ABCG2*) was identified as yet another ABC transporter that is overexpressed in anthracycline resistant cell lines.⁵⁶ Upon cloning of its cDNA, it turned out to be a half transporter—that is a protein consisting of only six transmembrane helices and one nucleotide binding domain compared with the classic duplicate motif. Other members of the ABCG subfamily (as well as some members from other subfamilies) are also half transporters. In general, these half transporters are believed to dimerise, either as homodimers or as heterodimers, into functional pumps.

Transfection of the *ABCG2* cDNA in cells confers resistance against mitoxantrone, topotecan, doxorubicine, daunorubicine, and rhodamine 123.⁵⁶ Using monoclonal antibodies against the *ABCG2* gene product, Maliepaard *et al*⁵⁷ recently demonstrated that this protein is present in the plasma membrane of endothelial cells and in the apical membrane of intestinal epithelial cells (both colon and small intestine) and hepatocytes. The latter strongly suggests that it is also involved in biliary secretion of amphipaths. Using a specific inhibitor of BCRP, GF120918, Jonker *et al*⁵⁸ showed that this transporter reduces the oral bioavailability of topotecan and other BCRP substrates in the intestine by pumping these compounds back into the lumen. Thus, BCRP as well as MDR1 and MRP2 are important in reducing the oral bioavailability of drugs and toxins.

CHOLESTASIS

Genetic forms of cholestasis

It has been known for a long time that a group of paediatric patients exists, who suffer from an inherited form of progressive intrahepatic cholestasis. The first report identified this disease entity in an Amish family (the Byler family), where seven members of four related sibships suffered from the same symptoms.⁵⁹ These children presented with steatorrhoea, diarrhoea, jaundice with intermittent exacerbations, hepatosplenomegaly, and failure to thrive. The outcome of the disease was generally fatal because of liver failure within the first decade of life. The advent of liver transplantation for children provided the opportunity to cure these patients. In further studies biochemical and histological features provided support for heterogeneity of this disease entity, although the clinical development was very similar. First and foremost the group fell into two parts, one subgroup of patients with a high serum γ -glutamyltransferase (GGT) activity and the other with a normal serum GGT activity.^{60,61} The latter group includes patients from the Byler pedigree. Liver histology of patients with high GGT PFIC revealed prominent bile duct proliferation and cirrhosis, which was absent or much less prominent in patients with low GGT. In the past few years considerable progress was made in identifying the genetic background of this group of patients, although it must be emphasised that not all involved genes have been identified yet. There are at least three groups that are now commonly designated by PFIC type 1, type 2, and type 3. Type 1 and type 2 patients have a low serum GGT activity, while type 3 patients have a high serum GGT activity.

Progressive familial intrahepatic cholestasis type 1 (formerly called Byler's disease)

Byler's disease primarily manifests itself as a chronic intrahepatic cholestasis. Bile salt concentrations are high in serum and very low in bile, suggesting that hepatic transport is impaired. In addition patients with PFIC type 1 suffer from watery diarrhoea. The jaundice in these patients is regarded as a secondary consequence of insufficient bile flow, the latter being dependent on bile salt secretion. With the patients from

the Byler pedigree and their family members a genetic screen was performed to identify the disease locus.⁶² This was located on chromosome 18q21-q22, the same chromosomal region on which a very similar disease type, benign recurrent intrahepatic cholestasis was localised (see below). A combined search for the two disease loci led to the identification of the mutated gene, *FIC1* (gene code *ATP8B1*), and its mutations in a group of patients, including those from the original Byler pedigree.⁶³ Many issues concerning the function of the *FIC1* gene product remain puzzling, however. Expression of *FIC1* in liver is actually quite low, while the gene is highly expressed in intestine and pancreas.⁶³ Expression is also found in many other tissues. Thus, it is unclear why the absence of *FIC1* from many tissues leads primarily to a phenotype in liver and intestine. On the basis of sequence homology, this gene encodes a P-type ATPase. Within the family of P-type ATPases, subgroups of transporters with different functions have been identified, including ion pumps and phospholipid flippases. Recently, Ujhazy *et al* expressed *FIC1* in CHO cells and observed increased translocation of NBD-aminophospholipid analogues from the outer to the inner leaflet of the plasma membrane. This suggests that *FIC1* is an inward flippase for aminophospholipids. How a lack of this function relates to the development of cholestasis remains to be explained.

Interestingly, interruption of the enterohepatic circulation (chronic bile diversion) was reported to dramatically improve the clinical picture of patients with low GGT PFIC. Whittington *et al*⁶⁴ described a procedure in which chronic external partial bile diversion was achieved through a jejunal stoma in four patients. In these patients pruritus dramatically improved and serum bile salt levels fell from >200 μM to less than 10 μM . Several later studies have reported similar results.⁶⁵⁻⁶⁷ This did not occur in all patients, however, and it would be of great interest to distinguish the beneficial effect of this procedure in PFIC type 1 compared with type 2 patients (see below).

Benign recurrent intrahepatic cholestasis (BRIC)

With the identification of the disease locus for PFIC type 1 and the responsible gene, *FIC1*, it became clear that the same gene is mutated in BRIC. BRIC most probably represents a mild form of PFIC type 1. PFIC type 1 may start with bouts of cholestasis and progressively develops into chronic persistent cholestasis, but in BRIC patients the phenotype remains restricted to periods of cholestasis that resolve after days to months. Importantly, when cholestasis resolves it leaves no detectable liver damage. The main features of BRIC are increased serum bile salt concentrations, jaundice and pruritus. The milder phenotype of BRIC compared with PFIC type 1, seems to correlate with the mutations found in these two patient groups.⁶³ DNA sequencing of the *FIC1* gene learned that deletions, frame shifts, and nonsense mutations appear to lead to the PFIC type 1 phenotype, while in BRIC patients generally missense mutations are found. This permits the hypothesis that the *FIC1* protein with BRIC mutations may have residual activity, while the protein is absent or non-functional in PFIC type 1 patients.⁶³

Progressive familial intrahepatic cholestasis type 2

The phenotype of this form of PFIC is very similar to that of type 1. It was demonstrated by Strautnieks *et al*⁶⁸ that the disease locus of this subgroup of patients resides on chromosome 2q24. They subsequently found that these patients have mutations in the *BSEP* gene, which is present in this region.¹⁹ The fact that mutations in the *BSEP* gene lead to a virtual absence of bile salts from bile has led to the conclusion that *BSEP* is the main bile salt transporter in the canalicular membrane. This is supported by studies in which rat and murine *Bsep* were transfected in Sf9 cells and shown to transport several bile salts.^{18, 69} No data are available yet on the transport characteristics of human *BSEP*. Both nonsense, missense

mutations, and deletions in the *BSEP* gene were found in some patients with low GGT PFIC, and these are now described as having type 2 PFIC.⁶⁸ Very importantly, further screening by Strautnieks *et al*⁷⁰ seems to indicate that still another subgroup of patients exists in whom the disease locus does not localise either to the *FIC1* region or to the *BSEP* region. This would mean that yet another gene is involved in low GGT PFIC.

Progressive familial intrahepatic cholestasis type 3

As mentioned above this form of PFIC is fundamentally different from type 1 and 2 in that these patients have a high serum GGT activity. The onset of this disease is somewhat later than in the other two forms, but the histological picture is more severe; there is strong bile duct proliferation and cirrhosis. The most prominent features of the disease are portal hypertension, hepatosplenomegaly, jaundice, and pruritus. If untreated the disease develops into liver failure. The genetic background of this subgroup of PFIC patients was elucidated after it was found that mice with a disruption in the *Mdr2* gene, the murine orthologue of *MDR3*, develop a similar phenotype. This led Deleuze *et al*⁷¹ to investigate the possible involvement of this gene in PFIC. Subsequently, in a group of 31 patients with high GGT PFIC, 17 were found to have a mutation in the *MDR3* gene.^{72, 73} As the gene was not completely sequenced in all these patients it is not clear whether the remaining 14 patients have as yet unidentified mutations or that another gene might be involved in this form of PFIC. *MDR3* P-glycoprotein functions in the translocation of phosphatidylcholine, thereby facilitating the secretion of this phospholipid into bile. The secretion of phospholipid is of crucial importance in the protection of the cellular membranes of the biliary tree against the high concentrations of bile salt detergents.⁴³

Treatment of patients with ursodeoxycholate seemed to be beneficial in about half of the cases.⁷² Given the fact that the hepatic damage is caused by bile salts, this is a rational treatment, because ursodeoxycholate is a hydrophilic bile salt that has low cytotoxicity. Indeed, in *Mdr2*^{-/-} mice with the equivalent defect, feeding of this bile salt also halted the disease process.^{74, 75} The observation that not all patients improve with ursodeoxycholate treatment may be explained by the fact that administration of this bile salt insufficiently replaces the endogenous bile salt pool. Thus, in patients who have no *MDR3* Pgp activity at all, the reduction of bile salt cytotoxicity by ursodeoxycholate is insufficient, while in patients with residual phospholipid secretion, ursodeoxycholate might bring the overall bile salt cytotoxicity below a critical threshold. Indeed, none of the patients with a truncated *MDR3* gene improved with ursodeoxycholate, while some of those with missense mutations did.⁷²

Interestingly, it became clear more recently that defects in the *MDR3* gene do not only give rise to paediatric liver disease. Jacquemin *et al* reported⁷⁶ that the mother of a patient with PFIC type 3 and several other women from this family suffered from intrahepatic cholestasis of pregnancy. These women turned out to be heterozygotes for the mutation in the *MDR3* gene, that caused PFIC type 3 in the homozygous index patient. Moreover, Rosmorduc *et al*⁷⁷ reported on six gallstone patients, in whom mutations in the *MDR3* gene were found. This included homozygous and heterozygous missense mutations as well as a heterozygous 1 bp insertion leading to frame shift and truncation. None of these mutations were observed in >100 chromosomes from control subjects suggesting that they may be associated with the disease phenotype. These two publications suggest that, apart from the severe phenotype associated with complete or nearly complete absence of *MDR3* function, also milder phenotypes exist that are associated with reduction but not complete absence of *MDR3* Pgp function.

Cholestasis of pregnancy

Intrahepatic cholestasis of pregnancy (ICP) is a reversible form of cholestasis that may develop in the third trimester of pregnancy and usually rapidly resolves after delivery. The incidence of ICP lies between 10 and 100 cases per 10 000 pregnancies, but there is a strong ethnical background to this phenomenon. Notably, in the Chilean population ICP develops in as much as 16% of pregnancies and within the Araucanian Indian subpopulation it is even as high as 28%.⁷⁸ The main symptoms are pruritus and, to a less extent, jaundice. Serum bile salt levels are increased.^{79–81} Increased incidence of fetal distress, premature birth, and even stillbirth in association with ICP has been reported (for review see Lammert *et al*⁸²).

Jacquemin *et al*⁷⁶ reported on the coexistence of PFIC type 3 and ICP in a consanguineous family. In this family, six women had at least one episode of ICP and of these six, four were heterozygous for the mutation in the *MDR3* gene. DNA of the other two was not analysed. These data suggest that a reduced *MDR3* Pgp activity predisposes for the development of ICP. A predisposition for ICP is not only observed with *MDR3* mutations; already in the original description of the Byler syndrome by Clayton *et al*⁸³ it was noted that the mother of a patient with this inherited disease (now characterised as a defect in the *FIC1* gene, see above) suffered from ICP. This has subsequently been confirmed in later studies.^{83, 84} Pregnancy may also unmask hitherto undiagnosed Dubin Johnson syndrome, caused by mutations in the *MRP2* gene.^{85–87} All in all, these data suggest that during pregnancy there is a more or less generalised reduction in bile formation and, combined with a pre-existing subclinical defect in any of these transport systems, this may induce clinical symptoms of cholestasis. The mechanism of reduced bile formation during pregnancy is unknown but the prevailing hypothesis is that it is related to the high levels of circulating hormones during the last trimester. The most important observation in support of this hypothesis is that the use of oral contraceptives may also induce intrahepatic cholestasis. Indeed, women with a history of ICP are also prone to cholestasis induced by oral contraceptives and vice versa.⁸⁸ Which hormones or hormonal metabolites are responsible is also unclear. In vivo experiments in rats suggest that conjugated oestrogen metabolites can be cholestatic, but progesterones may also play a part as these are produced at high rates during the last phase of pregnancy.⁸² These hormones may have an inhibitory effect on canalicular transporters, but as several transport processes are affected, it is also possible that the membrane composition is changed or that there is a generalised reduction in expression of transporters or an increased degradation.

Cholestasis associated with inflammation and sepsis

Sepsis is associated with impaired biliary secretion. Patients with sepsis often develop a conjugated hyperbilirubinaemia, suggesting that the secretion of bilirubin into bile is compromised.⁸⁹ These effects have been mimicked in many studies with rodents by injection of LPS and these studies indicate that *Mrp2* is strongly downregulated under these conditions. Roelofsen *et al*⁹⁰ demonstrated reduced bilirubin secretion into bile in rats at 16 hours after LPS injection and Vos *et al*⁹¹ subsequently showed that under these conditions *Mrp2* expression is very strongly downregulated. Recently, it became clear that members of the nuclear receptor family play a crucial part as transcription factors in the regulation of expression of many of the discussed transporter genes, including *MRP2*. Nuclear receptors function as heterodimers that regulate gene transcription in the presence of their cognate ligands.⁹² *MRP* seems to be regulated by several of these receptors (CAR and PXR/SXR) that dimerise with the retinoid X receptor, RXR.⁹³ Expression of the RXR is strongly downregulated during endotoxaemia in hamsters and this will lead to downregulation of *Mrp2*.⁹⁴ Expression of the bile

salt pump, BSEP, is also regulated by a nuclear receptor dimer, namely RXR:FXR.^{95, 96} In addition, expression of NTCP, which is involved in bile salt uptake by hepatocytes, is regulated by the dimer RXR:RAR.⁹⁷ Hence the expression of both NTCP and BSEP may also be prone to downregulation during sepsis. Studies with rodents have shown that sepsis indeed leads to a dramatic downregulation of Ntcp expression but has only a moderate effect on Bsep expression. These phenomena have so far been insufficiently studied in humans. It is interesting, however, that a recent report by Hinoshita *et al*⁹⁸ demonstrated a significant reduction in *MRP2* expression in patients with HCV infection that correlated with severity of the disease. In addition Zollner *et al*⁹⁹ observed decreased BSEP and *MRP2* protein levels in patients with PBC. Further studies in patients with several forms of hepatic inflammation and sepsis will have to demonstrate to what extent downregulation of hepatic transporter expression contributes to cholestasis.

Pruritus

Pruritus is a very prominent feature of cholestasis. The pathophysiological mechanism of cholestasis associated pruritus is still unknown. Indirect evidence exists for the association of pruritus with high levels of opioids (encephalins). Indeed, experiments with cholestatic rats demonstrated increased plasma enkephalin levels and treatment of cholestatic pruritic patients with opioid antagonists such as naltrexone has relieved itching in many but not all cases.¹⁰⁰ On the other hand, pruritus is thought to be associated with high serum and tissue bile salt concentrations, but a quantitative relation between peripheral bile salt levels and the severity of pruritus has never been established.¹⁰¹ It is interesting to note, however, that rifampicin was shown to relieve cholestasis associated pruritus in several cases.¹⁰² It was found recently that rifampicin is a ligand for the nuclear receptor SXR/PXR and ligand activation of this receptor induces expression of the cytochrome P450 isoforms that are capable of detoxifying hydrophobic bile salts.^{103, 104}

Hypercholesterolaemia of cholestasis

It is well known that patients suffering from chronic cholestasis develop hypercholesterolaemia. The plasma lipoprotein profile of cholestatic patients shows an abnormal lipoprotein fraction in the LDL region. This abnormal "cholestatic" lipoprotein was characterised as a unilamellar vesicle with an aqueous lumen, and was designated lipoprotein X (LpX).¹⁰⁵

In several studies on patients with low GGT PFIC, it was observed that these patients have a normal plasma cholesterol concentration compared with other forms of cholestasis.^{99, 106, 107} Very recently, this was also reported for PFIC type 3 patients.⁷² Using *Mdr2*^{-/-} mice, the animal model for PFIC type 3, it could be shown that the formation of LpX depends on the function of canalicular secretion machinery.¹⁰⁸ Wild type mice develop hypercholesterolaemia upon bile duct ligation and this is associated with the presence of massive amounts of LpX in the plasma, but LpX was completely absent in plasma of *Mdr2*^{-/-} mice.¹⁰⁸ Apparently, during cholestasis the formation of biliary vesicles continues and the release of these vesicles is redirected to the plasma compartment. Indeed, it was shown that during bile duct ligation, the expression of *Bsep* as well as *Mdr2* are not down regulated and that the proteins are redistributed from the canalicular membrane into an intracellular, subapical compartment, where they may continue to function in the formation of biliary vesicles. The conclusion from these data is that during obstructive cholestasis the continued formation of lipid vesicles by *Mdr2*/*MDR3* Pgp and the release into plasma leads to a dysregulation of cholesterol homeostasis.

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