Chapter 1

Function and regulation of hepatic transporters involved in bile flow

Jacqueline R.M. Plass, Peter L.M. Jansen, and Klaas Nico Faber

Division of Gastroenterology and Hepatology, University Medical Center Groningen, Groningen, The Netherlands
1.1 General introduction
The liver is regarded the chemical factory of our body. One central function is the production of bile. Bile is crucial for digestion as well as the clearance of hydrophobic toxic compounds. The liver is equipped with specialized transporter proteins that are responsible for maintaining a constant flow of bile to digestive tract. Malfunctioning of these transporters is the cause of cholestasis and jaundice. This chapter gives an overview of the function and regulation of the transporters involved in bile formation and their malfunctioning during inherited and acquired cholestatic diseases.

1.1.1 The liver
The liver is the second largest organ of the body and in human weighs approximately 1.5 kilograms.

Fig. 1-1 Schematic overall view of the blood flow and bile flow from and to the liver. Nutrient-rich blood from the intestine and oxygen-rich blood enter the liver via the portal vein and hepatic artery, respectively. Blood leaves the liver via the hepatic vein. Bile, produced by the liver, is stored in the gallbladder and is secreted into the intestine via the common bile duct. Arrows indicate the flow of blood or bile.

Approximately 75% of the blood supply to the liver is accounted for by the portal vein coming from the small intestine, stomach, pancreas and spleen. The other 25% flows through the hepatic artery. The blood leaves the liver via the hepatic vein (Fig. 1-1). The liver is involved in carbohydrate metabolism, fat and lipid metabolism, protein metabolism, transformation of drugs and vitamins, and detoxification. The
Chapter 1: Function and regulation of hepatic transporters involved in bile flow

Liver produces bile, which helps digestion and secretion of waste products. It is collected from the hepatic bile ducts in the gall bladder and is secreted into the intestine via the common bile duct (Fig. 1-1). The liver plays a role in glycogenogenesis and glycogenolysis; it stores glycogen and releases it on demand. The liver also stores other nutrients and vitamins. It synthesizes certain plasma proteins, like albumin (major contributor to colloid osmotic pressure of plasma), transporter of hormones, bilirubin, fatty acids, etc., fibrinogens (blood clotting) and other coagulation factors. It synthesizes lipoproteins, cholesterol and bile salts. In addition, the liver plays a role in immunological responses.

Fig. 1-2 Schematic view of the fine structure of the liver. The hepatocytes are arranged in plates. They form a physical barrier between the blood in the sinusoids and the bile in the canaliculi. The bile canaliculi are formed by the apical membranes of the hepatocyte. The sheet of endothelial cells, lining the sinusoid, is fenestrated for the passage of small molecules between blood and hepatocyte. Kupffer cells, stellate cells, and pit cells are also present in the sinusoids. The stellate cells are located in the space of Disse. This space between the endothelial wall and the hepatocytes contains and drains tissue fluid. The bile canaliculi merge into the larger bile ducts, which are lined by the cholangiocytes. The direction of blood flow and bile flow are indicated by arrows and arrowhead, respectively.

The liver consists of different cell types: parenchymal cells (hepatocytes), endothelial cells, Kupffer cells (macrophages), stellate cells (fat-storing and/or fibrotic cells), pit cells (natural killer cells) and oval or progenitor cells (Fig. 1-2). The vast majority of the cells in the liver are the hepatocytes. These cells carry out the primary functions of the liver: formation of bile, detoxification, and synthesis of blood proteins.
1.1.2 The liver units

There are three ways for dividing the liver in functional units: (1) the "classic" liver lobule, (2) the portal lobule, and (3) the hepatic acinus (Fig. 1-3).

Traditionally, the liver was divided in "classic" liver lobules, based on macroscopic morphology. The liver lobule is a hexagonal structure surrounded by connective tissue. Branches of the central vein lay in the center of the liver lobule. Hepatocytes are arranged in layers that radiate from the central vein to the portal triads on the corners, which consist of branches of the portal vein, the hepatic artery and a bile duct. The liver lobule unit emphasizes the drainage of blood by the central vein. The portal lobule unit places the portal triad in the center of a triangle of which the corners are branches of the central vein (Fig. 1-3) and emphasizes the blood supply by the portal vein and hepatic artery and the bile drainage by the bile duct. Bile, formed by the hepatocytes and secreted into the bile canaliculi is collected into bile ductules and drains into the bile duct of the portal triad. The third classification is that of the hepatic acinus. The boundaries of this diamond-shaped structure are formed by two branches of the central vein and the portal triads of two adjacent liver lobules. This unit emphasizes the secretory function of the liver.
Chapter 1: Function and regulation of hepatic transporters involved in bile flow

1.1.3 The hepatocyte

Hepatocytes are the main functional cells of the liver. They are responsible for the formation of bile, the metabolism of carbohydrates, proteins, fats, lipids, drugs and toxins, the synthesis of cholesterol, lipoproteins and plasmaproteins, detoxification, and storage of different substances like glycogen, fat and vitamins. Hepatocytes are especially enriched in smooth and rough endoplasmic reticulum, ribosomes, mitochondria, peroxisomes and lysosomes to adequately perform the many liver functions. Hepatocytes are polarized epithelial cells forming a physical barrier between sinusoidal blood and bile. The basolateral membrane is in contact with the sinusoidal blood and is the site for uptake of compounds. The apical membranes of adjacent cells form a lumen: the bile canaliculus. Tight junctions divide the plasma membrane in a basolateral domain and an apical domain. The vectorial transport of substances from sinusoidal blood to bile is mediated by specific transport proteins. A selection of these (human) proteins is shown in Fig. 1-4. Sinusoidal bile salt uptake is facilitated by NTCP and the OATP's in humans and Ntcp and Oatp's in rodents. The Na+/K+ATPase maintains the required electrochemical gradient. Biliary secretion of bile salts and other biliary compounds are carried out by BSEP, MDR3, MRP2, MDR1, ABCG5/G8, and FIC1 (in rodents these are Bsep, Mdr2, Mrp2, Mdr1a/1b, Abcg5/g5, and Fic1 respectively. The precise functions of these proteins are discussed later in this chapter.

Fig. 1-4 The hepatocyte houses specific transport proteins for mediating the vectorial transport from blood to bile of various compounds. Some of these are shown here. Bile salts are taken up from the sinusoidal blood by NTCP and the OATP's. The Na+/K+ATPase maintains the required electrochemical gradient. Bile salts are secreted into the bile canaliculus by BSEP and also MRP2. Bile salt secretion triggers the release of phosphatidylcholine and cholesterol, which are supplied by MDR3 and ABCG5/G8 respectively. The best-known ABC transporter, MDR1, is involved in the elimination of bulky amphiphatic organic anions. Under cholestatic conditions, members of the ABCC family, the MRPs, are up-regulated and may function as an overflow system for bile salts.

NTCP Na+/K+ATPase OATP's MRP's

Hepatocyte Bile canaliculus

MRP2 ABCG5/G8

BSEP

FIC1

MDR3

MDR1

Bile canaliculus
1.1.4 Bile

The formation of bile is an important function of the liver. Bile is a body fluid, mainly containing water, electrolytes, and organic molecules including bile salts, phospholipids, cholesterol, and bilirubin. It flows through the biliary tract and via the gallbladder (except in rat) into the intestine. Bile secretion into the intestine has two main functions: (1) bile salts are important for digestion of dietary lipids and uptake of fat-soluble vitamins (A, D, E, and K); (2) it is the major route for the elimination of surplus cholesterol, waste products, bilirubin, drugs and other toxic components.

Bile secretion is an osmotic process. The major driving force for bile formation is the secretion of bile salts at the canalicular membrane of the hepatocyte, the so-called bile salt-dependent bile flow. Canalicular secretion of reduced glutathione (GSH) also contributes to bile flow, the bile salt-independent bile flow.

The biliary tract begins in the liver: the hepatocytes secrete bile into the bile canaliculi, these canaliculi merge into the bile ducts of the portal triad. The bile ducts merge into the common bile duct that allows bile to drain directly into the duodenum when it is needed for digestion. During fasting, bile is temporarily stored and concentrated in the gallbladder. Storage in the gallbladder is, however, redundant since a gallbladder is completely absent in rats and in humans it may be removed without any consequence.

\[\text{Neutral pathway} \quad \text{Acidic pathway}\]

\[\text{Cholesterol} \xrightarrow{7\alpha\text{-hydroxylase}} 7\alpha\text{-hydroxycholesterol} \xrightarrow{7\alpha\text{-hydroxycholesterol} \text{ 27\alpha\text{-hydroxylase}}} \text{cholic acid} \quad \text{chenodeoxycholic acid} \]

Fig. 1-5 The two pathways of bile salt synthesis: the classic pathway and the alternative pathway. The classic pathway starts with the conversion of cholesterol into 7α-hydroxycholesterol in the endoplasmic reticulum, while the alternative pathway starts in the mitochondrion with the conversion into 27α-hydroxycholesterol. Both pathways end in the peroxisomes resulting in the primary bile salts cholic acid and chenodeoxycholic acid.

1.1.5 Bile salts

Bile salts are exclusively synthesized in the liver and are formed as natural end products of cholesterol metabolism. They are amphiphatic molecules, which means that bile salts have both water-soluble (hydrophilic) and water-insoluble (hydrophobic sides) characteristics. This dual nature enables bile salts to carry out their function.
Chapter 1: Function and regulation of hepatic transporters involved in bile flow

Bile salts facilitate the emulsification of lipids and subsequently help the actual digestion by lipases. In addition, bile salts help to solubilize and transport lipids by forming so-called micelles. Micelles are composed of bile salts and lipids (fatty acids, cholesterol, and monoglycerides) that are water-soluble, thus enabling transport of lipids and a great variety of other hydrophobic compounds.

In humans, the two most abundant bile salts are cholic acid (CA) and chenodeoxycholic acid (CDCA), which are referred to as primary bile acids. The primary bile acids are synthesized from cholesterol either via the classic (also called the neutral) pathway, which is also the predominant pathway in human, or the alternative (also called the acidic) pathway (Fig. 1-5). The first step of the classic pathway is established by the enzyme cholesterol 7α-hydroxylase and involves a modification of the ring structure. This reaction takes place in the endoplasmic reticulum. The first step of the alternative pathway takes place in the mitochondrion, where the enzyme sterol 27-hydroxylase modifies the sterol side chain. The classic pathway produces both cholic acid and chenodeoxycholic acid, while the alternative pathway produces predominantly chenodeoxycholic acid. Some enzymatic conversions take place in the cytosol. The final reactions in the formation of the primary bile acids occur in peroxisomes. Prior to excretion into bile, the bile acids are conjugated to taurine or glycine and are now referred to as bile salts. The ultimate fate of the conjugated bile salts is secretion into the intestine. There, approximately 95% of the conjugated bile salts are reabsorbed and are transported back to the liver via the portal blood circulation. In the liver, they are taken up again by the hepatocytes for re-secretion into bile. This recycling process is referred to as the enterohepatic circulation of bile salts. Every day, approximately 4 grams of bile salts cycle about 6 times between liver and intestine. And although this enterohepatic circulation of bile salts is very efficient, 5% (approximately 1 gram per day) is lost each cycle via fecal secretion. This loss is compensated for by de novo synthesis by the liver.

As discussed before, the primary function of bile salts is their role as detergents to keep dietary fats and fat-soluble vitamins available for metabolism, and to remove hydrophobic compounds from the body, including cholesterol and toxins. In recent years, however, bile salts have been shown to perform several crucial signaling functions in the mammalian cell as well. For example, they function as highly selective mediators of gene transcription. This is accomplished by binding and activation of the transcription factor Farnesoid X Receptor (FXR). Many genes, involved in bile salt, cholesterol and lipoprotein metabolism are regulated by bile salt-activated FXR(see section 1.5.2). In addition, bile salts are now known to play an important role in regulation of apoptosis and in intracellular signal pathways involving protein kinase C isoforms and phosphatidylinositol-3 kinase as well.
### Table 1-1. Hepatic transporters involved in bile flow. Indicated are their localization, substrates, relation to inherited diseases, regulation during acquired cholestasis, and transcriptional regulation.

<table>
<thead>
<tr>
<th>Name</th>
<th>Loc.</th>
<th>Substrates</th>
<th>Inherited disease</th>
<th>Exp. Chol.</th>
<th>Transcriptional regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTCP/Ntcp (SLC10A1/Slc10a1)</td>
<td>SM</td>
<td>Bile salts</td>
<td>↓</td>
<td>↓</td>
<td>RARα, HNF1α, HNF4α(?), C/EBP, HEX</td>
</tr>
<tr>
<td>OATP-C (SLC21A6)</td>
<td>SM</td>
<td>Organic anions, Bile salts, Bilirubin (conjugates)</td>
<td></td>
<td></td>
<td>HNF1α</td>
</tr>
<tr>
<td>OATP-A (SLC21A3)</td>
<td>SM</td>
<td>Organic anions, Bile salts, Bulky organic cations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OATP8 (SLC21A8)</td>
<td>SM</td>
<td>Organic anions Bile salts(?)</td>
<td></td>
<td></td>
<td>FXR, HNF1α</td>
</tr>
<tr>
<td>OATP-B (SLC21A9)</td>
<td>SM</td>
<td>Organic anions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oatp1 (Slc21a1)</td>
<td>SM</td>
<td>Bile salts, Organic anions/cations</td>
<td>↓</td>
<td></td>
<td>HNF4α(?) HNF1α</td>
</tr>
<tr>
<td>Oatp2 (Slc21a5)</td>
<td>SM</td>
<td>Bile salts, Organic anions/cations</td>
<td>↓</td>
<td>↓</td>
<td>PXR HNF1α</td>
</tr>
<tr>
<td>Oatp4 (Slc21a10)</td>
<td>SM</td>
<td>Organic anions Bile salts</td>
<td>↓</td>
<td></td>
<td>HNF1α</td>
</tr>
<tr>
<td>BSEP/Bsep (ABCB11/Abcb11)</td>
<td>CM</td>
<td>Bile salts</td>
<td>PFIC2 BRIC2</td>
<td>↔/↓</td>
<td>FXR HNF4α(?)</td>
</tr>
<tr>
<td>MRP2/Mrp2 (ABCC2/Abc2)</td>
<td>CM</td>
<td>Bilirubin conjugates Anti-cancer drugs</td>
<td>Dubin-Johnson</td>
<td>↓</td>
<td>FXR, RARα C/EBPβ PXR, CAR</td>
</tr>
<tr>
<td>MDR3/Mdr2 (ABCB4/Abcb4)</td>
<td>CM</td>
<td>Phosphatidylcholine</td>
<td>PFIC3 ICP</td>
<td>↔</td>
<td>FXR, PPARα HNF4α(?), SP1</td>
</tr>
<tr>
<td>ABCG5/G8 Abcg5/g8</td>
<td>CM</td>
<td>Cholesterol</td>
<td>Sito-sterolemia</td>
<td>↑</td>
<td>LXRα LXRβ</td>
</tr>
<tr>
<td>MDR1 / Mdr1a/1b</td>
<td>CM</td>
<td>Bulky amphipatic organic anions</td>
<td>Anti-cancer drugs</td>
<td>↑/↑</td>
<td>PXR SP1</td>
</tr>
<tr>
<td>FIC1/Fic1 (ATP8B1/Arp8b1)</td>
<td>CM</td>
<td>Aminophospholipids(?) Bile salts(?)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


↑: expression is up-regulation; ↔: expression is unaltered; ↓: expression is down-regulated
Chapter 1: Function and regulation of hepatic transporters involved in bile flow

1.2 Hepatic bile salt transport proteins
The formation of bile is driven by the vectorial transport of solutes from the sinusoidal blood into the hepatocyte and from the hepatocyte into the bile canaliculus. Since hepatocytes form a physical barrier between sinusoidal blood and canalicular bile, the transport of compounds from blood to bile is carried out by specialized carrier systems. The hepatocyte houses various proteins, located in the sinusoidal and canalicular membranes, which mediate this transport (Table 1-1).

1.2.1 Basolateral or sinusoidal transport proteins
The hepatocyte is equipped with several transport proteins to extract metabolites, drugs and other compounds from the blood circulation. Sinusoidal uptake of compounds is mediated by members of the superfamily of solute carriers (SLC). SLC transport proteins are passive transporters, ion coupled transporters or exchangers (Fig. 1-6). These proteins use the electrochemical gradient to facilitate transport of solutes across the membrane. Currently, the superfamily of human solute carriers comprises 43 families and 298 genes (http://www.bioparadigms.org/slc/intro.asp). A member of a specific SLC family is assigned to that family if it has a protein sequence homology of at least 20-25%.

Fig. 1-6 Members of the SLC superfamily and members of the ABC transporter family are involved in the transport of compounds across the sinusoidal and apical membranes of the hepatocyte. The SLC transporter family comprises ion coupled transporters (1), exchangers (2), and passive transporters (3). ABC transporters need the energy from ATP hydrolysis for transport and are therefore active transporters (4). (Adapted from11)

In the basolateral membrane of the hepatocyte, two families are important for bile salt uptake: (1) SLC family 10, the sodium bile salt cotransport family with 6 members;12 and (2) SLC family 21 or O, the organic anion transport family, with 11 members.13 The Na+-dependent taurocholate cotransporting polypeptide (NTCP in human, Ntcp in rodents; SLC10A1 according to SLC nomenclature) is the major hepatic bile salt
importer.\textsuperscript{14,15} It is exclusively expressed in the liver,\textsuperscript{15} where it is present at the basolateral membrane of the hepatocyte.\textsuperscript{16} Its membrane topology predicts 7 membrane spanning regions, with the N-terminus outside and the C-terminus inside the cell\textsuperscript{14}. NTCP preferentially transports conjugated bile salts\textsuperscript{17} and are transported in a Na\textsuperscript{+}-dependent manner via cotransport of Na\textsuperscript{+}. The Na\textsuperscript{+}/K\textsuperscript{+}-ATPase maintains the Na\textsuperscript{+}gradient required for this transport. Recently, it has been shown for rat Ntcp that it is also able to transport sulfobromophthalein in a Na\textsuperscript{+}-dependent manner.\textsuperscript{18} Sulfobromophthalein is an organic anion that is used as a diagnostic tool in the assessment of hepatic function. Thus, the substrate specificity of Ntcp is possibly not as narrow as previously considered.

The Na\textsuperscript{+}-independent uptake of bile salts is mediated by members belonging to the SLC21 or SLCO family (the Organic Anion-Transporting family), also known as the OATP's. Four OATP's are expressed in human hepatocytes: OATP-C, OATP8, OATP-B, and OATP-A. The first three are (possibly) involved in bile salt uptake. OATP-C (or SLC21A6) is exclusively expressed in liver\textsuperscript{19,20} and was initially designated as the liver-specific organic anion transporter. This protein is the major Na\textsuperscript{+}-independent bile salt importer and also transports unconjugated bilirubin and its conjugates\textsuperscript{21}. OATP-A (SLC21A3) is expressed in many tissues, including liver, but is most important in the blood-brain barrier and transports organic anions, bile salts, and bulky organic cations.\textsuperscript{22} OATP-8 (SLC21A8) expression is, like that of OATP-C, restricted to the liver.\textsuperscript{23} There is some controversy whether this protein transports bile salts or not.\textsuperscript{24} OATP-B (SLC21A9) is expressed in various tissues, with the highest expression in the liver, but there is no experimental evidence that it transports bile salts.\textsuperscript{24}

In rodents, three Oatp’s are described to be involved in the Na\textsuperscript{+}-independent uptake of bile salts: (1) Oatp1 (Slc21a1) which is expressed in liver, kidney and choroid plexus\textsuperscript{25}; (2) Oatp2 (Slc21a5) is also expressed in liver and kidney,\textsuperscript{25,26} and (3) Oatp-4 (Slc21a10) which is expressed only in the liver.\textsuperscript{27} Under cholestatic conditions when serum bile salt levels are high, the expression of members of the ABCC family is induced to protect the hepatocyte by exporting bile salts.\textsuperscript{28-31} From this protein family, Multidrug Resistance Proteins 1, 3, and 4 are located in the sinusoidal membrane and have been shown to be capable of transporting bile salts.\textsuperscript{32-35} Concurrently, these proteins are proposed to function as an overflow efflux system for accumulated bile salts during cholestasis.

\subsection*{1.2.2 Canalicular bile salt transport}

Transport from the hepatocyte across the canalicular membrane occurs against a steep concentration gradient. It is predominantly mediated by members of the Adenosine Triphosphate (ATP)-binding cassette (ABC) transporter superfamily. Contrary to the SLC transporters, ABC transporters are active transporters: they couple cellular energy to the transport of substrates across membranes. In the case of ABC transporters, the energy comes from the hydrolysis of ATP (Fig. 1-6). A typical ABC transporter protein consists of two halves connected by a linker-peptide (Fig. 1-7). Each half contains a so-called nucleotide-binding domain and a transmembrane domain. The nucleotide-binding domain, located at the cytoplasmic side of the membrane, is the
Chapter 1: Function and regulation of hepatic transporters involved in bile flow

place for ATP binding and hydrolysis and is characterized by the presence of the conserved Walker A and B motifs and the signature or C motif.36 The transmembrane domain consist of 6 to 11 membrane spanning α-helices and determines the substrate specificity of the protein.37 Up to now, 48 human ABC transporters are known and divided in seven subfamilies, designated A to G (http://nutrigene.4t.com/humanabc.htm). Members of three of these subfamilies (B, C, and G) are involved in bile formation at the canalicular membrane.

![Fig. 1-7 Schematic structure of three different ABC transporters. Full transporters like MRP1 and BSEP contain two transmembrane domains and two NBD's. A half transporter like ABCG5 contains only one transmembrane domain and one NBD. The number of membrane spanning α-helices in the transmembrane domain may vary. For example, the N-terminally transmembrane domain of MRP1 contains 11 membrane-spanning α-helices. While the two transmembrane domains of BSEP contain 6 membrane spanning α-helices each.](image)

Conjugated bile salts are excreted into bile by the Bile Salt Export Pump (BSEP in human, Bsep in rodent; *ABCB11*; previously known as sister of P-glycoprotein or SPGP).38-40 BSEP belongs to the ABCB subfamily and is specifically expressed in the liver where it is the main determinant of bile salt-dependent bile flow. While BSEP transports monovalent bile salts across the canalicular membrane, the Multidrug
Resistance Related Protein 2 (MRP2 in human, Mrp2 in rodent; \textit{ABCC2}) is able to mediate the transport of sulfated and glucorinated bile salts.\textsuperscript{41,42} Besides the liver, it is also expressed in kidney and duodenum.\textsuperscript{43} MRP2 mediates the transport of many other glutathione-, gluconate-, and sulfate-conjugated anions, among which its primary function as transporter of conjugated bilirubin. In addition, it also transports anticancer drugs (hence its name) and reduced glutathione.\textsuperscript{44-47} MRP2 is the main determinant of the bile salt-independent bile flow.

In response to canalicular bile salt secretion, phosphatidylcholine and cholesterol are released from the outer leaflet of the canalicular membrane and, together with bile salts, form micelles, thereby protecting the bile duct epithelium from the detergent-effects of bile salts. The Multidrug resistance 3/2 protein (MDR3 in human and Mdr2 in rodents; \textit{ABCB4}) functions as a phospholipid flippase and mediates the transfer of phosphatidylcholine from the inner leaflet of the hepatocanalicular membrane to the outer leaflet.\textsuperscript{48,49} It is predominantly expressed in the liver, but also in muscle, heart, and spleen.\textsuperscript{50-52}

Surplus cholesterol is eliminated from the body via bile. On the one hand, through conversion of cholesterol to bile salts, and on the other hand, through direct transport from the hepatocyte to the bile. Two members belonging to the ABCG family, ABCG5 and ABCG8, are involved in biliary cholesterol transport.\textsuperscript{53,54} These proteins are so-called half transporters: they contain only one transmembrane domain (consisting of 6 membrane-spanning \(\alpha\)-helices and one NBD (Fig. 1-7). Half transporters need to form homodimers or heterodimers to be a functional transporter. ABCG5 and ABCG8 are expressed in liver and small intestine,\textsuperscript{55} and need to be co-expressed for canalicular targeting and biliary cholesterol secretion.\textsuperscript{56,57}

The best-known ABC transporter is a member of the ABCB subfamily: the Multidrug Resistance protein 1 or P-glycoprotein (MDR1 in human; mice and rats have two homologues: Mdr1a and Mdr1b). This transport protein was first designated as P-glycoprotein since its presence correlated with the altered drug permeability of mutant (drug resistant) Chinese hamster ovary cells.\textsuperscript{58} Transfection of the MDR1 gene is sufficient to generate multidrug resistant cells.\textsuperscript{59} MDR1 is expressed in the apical membranes of various tissues, such as the brain, kidney, intestine, placenta, and liver.\textsuperscript{50,60,61} In normal human liver, MDR1 is lowly expressed and is involved in the elimination of bulky amphiphatic organic anions, including various drugs.

Besides the ABC-transporters, the canalicular membrane contains also a P-type ATPase, FIC1 or ATP8B1 that is involved in bile salt homeostasis. FIC1/ATP8B1 is expressed in liver (canalicular membrane of hepatocytes, apical membrane of cholangiocytes) and pancreas, but to a much higher level in the small intestine (brush border\textsuperscript{62,63}). The precise function of FIC1 is not clear yet. It has been suggested that it is a translocator of amino-phospholipids.\textsuperscript{63} The suggestion that FIC1 is involved in the transport of bile salts\textsuperscript{62} has been contradicted by a recent study from Harris et al.\textsuperscript{64} that suggests that FIC1 does not function as a bile salt transporter, so the precise function of this protein remains elusive. However, FIC1 does affect bile salt homeostasis. For example, loss of the murine \textit{Atp8b1} gene\textsuperscript{65} results in elevated serum bile salt levels. In addition, absence of human FIC1 is associated with reduced FXR, the bile salt sensor.\textsuperscript{66,67}
1.2.3 Intracellular bile salt transporters

Bile salts taken up from the sinusoids have to be transported across the hepatocyte to the canalicular membrane. However, little is known about this intracellular transport. It is assumed that it is not vesicular-, but carrier-mediated, involving bile salt binding proteins. In addition, membrane proteins are probably needed to transport bile salt intermediates during the multi-organellar processes of de novo bile salt synthesis. Enzymes involved in this process reside in the endoplasmic reticulum, mitochondria, cytoplasm, or peroxisomes, illustrating the need for transport systems. Little is known about the proteins involved in this process.

1.2.4 Other transporters involved in the enterohepatic circulation of bile salts

The enterohepatic circulation of bile salts requires that bile salts are reabsorbed from the intestinal lumen to the blood. The process takes place in the terminal ileum. Na+-dependent uptake of bile salts is accomplished by the ileal Apical Sodium-dependent Bile Acid Transporter (ASBT) in the apical membrane of enterocytes. In rodents, Oatp3 may add to the intestinal bile salt reabsorption through a Na+-independent process. In enterocytes, bile salts bind to the Ileal Bile Acid-Binding Protein (IBABP) and are transported across the basolateral membrane by the Ostα-Ostβ heterodimeric transporter.

1.3 Inherited diseases associated with hepatic transport functions

In recent years, important progress has been made in our understanding of the function of hepatic transport proteins and their role in development of cholestasis. The elucidation of the genetic defect of various inherited forms of progressive cholestasis has been instrumental to show the crucial function of various transporters in bile salt homeostasis.

1.3.1 Progressive Familial Intrahepatic Cholestasis

A severe type of cholestatic liver disease is Progressive Familial Intrahepatic Cholestasis (PFIC). PFIC is inherited in an autosomal recessive manner, and becomes manifest during early childhood, and, when untreated, may result in liver failure. At least three subtypes have been recognized: PFIC 1, 2, and 3.

1.3.2 Progressive Familial Intrahepatic Cholestasis type 1

PFIC1 was first called Byler’s disease, since the first PFIC patients described were descendants of the Amish Jacob Byler. PFIC1 is characterized by jaundice, severe itching, high serum bile salt concentration, low biliary bile salt concentration, and low serum γ-glutamyl transpeptidase (γ-GT) level. It is caused by mutations in the FIC1 or ATP8B1 gene, which has been mapped to chromosome 18q21-22. To date, 54 different mutations in the FIC1 gene have been documented to cause PFIC1. One of these mutations, G308V, was commonly detected in patients with Byler’s disease and was investigated further using a mouse model. Mice carrying this mutation display...
a milder form of cholestasis compared to PFIC1 patients carrying the same mutation.\textsuperscript{65} Despite extensive \textit{in vivo} and \textit{in vitro} experiments using human and mouse FIC1/Fic1, it is still unclear what the molecular function of the FIC1 protein is. The clinical features of PFIC1 clearly indicate a role in bile formation. However, FIC1 itself seems not capable of transmembrane transport of bile salts. Recently, Chen et al.\textsuperscript{66} showed a relationship between the loss of FIC1 and reduced activity of FXR. In addition, reduced FXR levels were also shown in a PFIC1 patient.\textsuperscript{67} FXR is a bile salt-activated transcription factor (see below) controlling the expression of genes encoding proteins involved in bile salt biosynthesis and transmembrane transport. Reduced FXR levels would lead to reduced canalicular secretion of bile salts by the bile salt export pump (BSEP), ultimately resulting in the cholestasis. However, the mechanism that is responsible for the specific decrease in FXR levels in the absence of FIC1 remains to be elucidated.

1.3.3 Benign Recurrent Intrahepatic Cholestasis type 1
Mutations in the \textit{ATP8B1} gene may also cause autosomal recessive inherited Benign Recurrent Intrahepatic Cholestasis type 1 or BRIC1.\textsuperscript{62,79} While PFIC1 presents in early infancy, BRIC manifests itself during adolescence and early adulthood and is characterized by recurrent episodes of cholestasis. Deletions, frame shifts and nonsense mutation appear to lead to PFIC1, while missense mutations, which could result in suboptimal protein expression/activity, appear to lead to BRIC.\textsuperscript{62} Intrahepatic cholestasis of pregnancy has also been associated with BRIC.\textsuperscript{80}

1.3.4 Progressive Familial Intrahepatic Cholestasis type 2
Some non-Amish PFIC patients, who displayed characteristics of PFIC1 patients of the Amish kindred, could not be mapped to the same locus as FIC1.\textsuperscript{81-83} In addition, morphological differences were found between these two groups of patients. PFIC1 patients have coarsely granular bile and bland intracanalicular cholestasis, while PFIC2 patients have amorphous bile and neonatal hepatitis.\textsuperscript{82} The PFIC2 locus on chromosome 2q24\textsuperscript{84} corresponds with the \textit{ABCB11} gene, encoding the Bile Salt Export Pump.\textsuperscript{85} \textit{In vivo} experiments support these findings. Disruption of the murine \textit{Abcb11} gene results only in mild intrahepatic cholestasis,\textsuperscript{86} but leads to severe cholestasis when combined with cholate-feeding.\textsuperscript{87} Several mutations have been described which lead to loss of ABCB11 expression.\textsuperscript{88} The missense mutations lead to disturbed trafficking, decreased expression, and defective or decreased transport.\textsuperscript{89-91} We have studied this for the aspartate to glycine mutation at position 482 that appears to result in a functional, but highly unstable and temperature-sensitive protein (chapter 3\textsuperscript{91}). Mutations like the D482G are clinically very interesting, because they may pave the way for new therapies, aimed at the restoration of expression of mutant -but functional- proteins at the correct subcellular location.
Chapter 1: Function and regulation of hepatic transporters involved in bile flow

1.3.5 Benign Recurrent Intrahepatic Cholestasis type 2

Recent studies show that the cause of benign recurrent intrahepatic cholestasis is not restricted to mutations in FIC1 but may also result from mutations in ABCB11 and other, yet unknown, genes. BRIC patients from 20 families were found to have no mutations in the FIC1 gene. Subsequent sequencing of the ABCB11 gene revealed that 11 patients (from 8 different families) contained mutations in this gene. Similar to BRIC1, BRIC2 is caused by missense mutations as well as one putative splice site mutation. Remarkably, one patient appeared to be homozygous for the E297G mutation that was previously reported to cause PFIC2. Thus, other genetic and/or environmental factors play a role in the symptoms associated with reduced BSEP function. This study also shows that there appears to be at least one more locus for autosomal recessive BRIC and PFIC with low serum GGT. In addition, there are indications that single nucleotide polymorphisms in the ABCB11 gene may be involved in intrahepatic cholestasis of pregnancy.

1.3.6 Progressive Familial Intrahepatic Cholestasis type 3

The third subtype, PFIC3, is different from type 1 and 2 in that serum γ-GT level is high, biliary phospholipids are absent, serum bile salt level is normal, and the presence of extensive bile duct proliferation, cirrhosis and fibrosis. The lack of biliary phospholipids pointed to an impaired MDR3 (ABCB4) function, the ABC-transporter that acts as a flippase for phospholipids. Indeed, PFIC3 patients lack MDR3 expression, due to mutations in the MDR3 gene. MDR3 mutations may also be a cause of intrahepatic cholestasis of pregnancy and of cholesterol gallstone disease.

1.3.7 Dubin-Johnson syndrome

The Dubin-Johnson syndrome is an autosomal recessive disorder with a relative benign character. This syndrome is not life threatening and no specific treatment is required. The syndrome was first described by Dubin, Johnson, Sprinz and Nelson, hence its name. The only symptom of Dubin-Johnson syndrome is a benign form of jaundice throughout the patient’s life. Patients with this disease lack the bilirubin transporter, MRP2 (ABCC2), due to mutations in the corresponding MRP2 gene. The secretion of conjugated bilirubin and other anionic conjugates to bile is impaired, but the transport of bile salts into bile is normal. The bilirubin(-conjugates) accumulate in liver and blood, causing the jaundice. The Dubin-Johnson syndrome is phenotypically similar to transport deficient (TR) rats, a strain that occurred naturally and lacks Mrp2 expression. Several mutations in the MRP2 gene have been described in Dubin-Johnson syndrome patients and in (TR-) rats. Some of the human MRP2 mutations were studied in detail. Dubin-Johnson-causing mutations may result in impaired transport activity, deficient maturation and/or impaired sorting of the transporter. The expression of another MRP isoform, MRP3 (ABCC3), is up-regulated when MRP2 function is impaired. MRP3 is located in the basolateral membrane of the hepatocyte and this up-regulation may compensate for the impaired canalicular secretion of harmful compounds.
1.3.8 Sitosterolemia

Sitosterolemia is an autosomal recessive lipid disorder. It is characterized by accumulation of plant sterols (mainly sitosterol, hence the name) in blood and tissues, due to enhanced sterol absorption, reduced biliary sterol secretion and reduced cholesterol synthesis. In 1974, this disorder was described by Bhattacharyya and Connor as a lipid storage disease in two affected sisters. Other clinical features are cholesterol deposits (xanthomas) in skin, tendons, and coronary arteries, leading to premature atherosclerosis. The disease is caused by mutations in the \(ABCG5/G8\) genes which are located at chromosome 2p21. ABCG5 and ABCG8 are half transporters and function as a heterodimer. This explains why in PFIC1 patients, mutations in one of the genes is enough to result in Sitosterolemia. Studies performed with mice that are defective of either Abcg5 or Abcg8 confirmed these observations. Indeed, loss of only Abcg5 is sufficient to result in symptoms of Sitosterolemia. In these mice, plant sterol concentrations in plasma are elevated. Similarly, disruption of Abcg8 leads to loss of biliary cholesterol secretion, although biliary sitosterol secretion appeared to be preserved.

1.4 Hepatic transporter regulation during acquired liver disease

The expression of transport proteins is also affected during acquired liver disease. These represent by far the more common causes of cholestasis. The hepatic responses to the different forms of acquired liver diseases generally serve to protect the hepatocyte against the toxic effects of accumulated bile salts and other bile compounds. Several acquired forms of liver disease can be distinguished: primary biliary cirrhosis, primary sclerosing cholangitis, inflammation-induced intrahepatic cholestasis (caused by sepsis, drugs, hormones, and alcohol), and extrahepatic biliary obstruction (caused by gallstones or tumors). Several animal models are used to study the expression of hepatic transporters during intrahepatic or extrahepatic cholestasis (for reviews: 2, 118). These models are detailed below.

1.4.1 Endotoxin-induced cholestasis

Sepsis is often associated with cholestasis. Lipopolysaccharide (LPS), a bacterial cell wall component, is the key mediator that is responsible for the development of cholestasis. It induces the release of inflammatory cytokines by the Kupffer cells in the liver, which, in turn, have major effects on protein expression in the hepatocyte. The endotoxin-treated rodent is an animal model to study this type of acquired liver disease. In the endotoxemic rat, the bile salt transport to the bile is strongly impaired, leading to cholestasis. This is associated with major effects on expression of bile salt uptake transporters in the hepatocyte. Ntcp and Oatp’s are simultaneously down-regulated, while expression of the bile salt export system, Bsep, is maintained. The multidrug resistance proteins are either maintained (Mdr1a and Mdr2) or elevated (Mdr1b and Mrp1).
Chapter 1: Function and regulation of hepatic transporters involved in bile flow

Another characteristic of sepsis is jaundice, probably as a consequence of impaired bilirubin transport. This is indeed explained by the rodent-model: in the endotoxemic rat, the bilirubin transporter Mrp2 is down-regulated.\textsuperscript{28,124} The regulation of transporter expression may be different between human and rodent. For example, in both human and rat MRP2, Mrp2 expression is down-regulated, but in humans this is accomplished post-transcriptionally, while in rat it is regulated at the transcriptional level.\textsuperscript{31,125,126} In addition, human BSEP expression is decreased in liver slices treated with LPS, while rat Bsep expression is unaltered after the same treatment.\textsuperscript{126}

1.4.2 Estrogen-induced cholestasis

Cholestasis may also be associated with pregnancy or caused by oral contraceptives. The animal model for studying this type of cholestatic condition is $17\alpha$-ethinylestradiol (EE)-treatment of rodents. In EE-treated rats, both sinusoidal and canalicular bile salt transport systems were affected. The protein levels of Ntcp, Oatp1, Oatp2, Oatp4, Bsep, and Mrp2 were all decreased.\textsuperscript{122,127,128} Mdr1a/b expression levels remain unchanged.\textsuperscript{122} As in endotoxin-induced cholestasis, Na$^+$/K$^+$ATPase activity was also reduced.\textsuperscript{129} Another important mechanism that effects efficient bile salt transport from the hepatocyte is the fact that the estrogen metabolite, estradiol-17beta-D-glucuronide, inhibits the Bsep transport activity directly. This may be an even more significant factor than alterations in expression levels.\textsuperscript{40} Bsep has a very high transport capacity. Reduction in Bsep protein levels is therefore not necessarily associated with reduction of the canalicular bile salt secretion rate.\textsuperscript{130}

1.4.3 Bile duct ligation

The animal models described above are models for intrahepatic cholestatic conditions. Ligation of the common bile duct is an animal model for extrahepatic obstructive cholestasis. The most common cause of extrahepatic obstructive cholestasis is the presence of gallstones. In extrahepatic obstructive cholestasis, bile ducts are blocked, leading to accumulation of bile in the liver, which in turn results in liver damage and eventually in liver failure.

Gap junctions disappear in bile duct-ligated rats,\textsuperscript{131} leading to an altered physical barrier between portal blood and bile. Expression of Oatp1, Oatp4, Bsep, Mrp2, and Mrp6 are down-regulated,\textsuperscript{29,121,122,127,132-134} while expression of Mdr1a, Mdr1b, Mrp3, and Mrp4 are up-regulated.\textsuperscript{29,30,122,135,136} Mrp3 and Mrp4 are both able to transport sulfated bile salts and their up-regulation may serve as escape route for bile salts in order to protect the hepatocyte from toxic levels of bile salts.\textsuperscript{29,33,136}

1.5 Transcriptional regulation of hepatic transport proteins

Expression of proteins involved in bile formation and homeostasis is tissue-specific with selective expression of liver-specific and intestine-specific transporters. In addition, the expression of these transporters is strictly regulated to fit the need to
maintain bile flow and formation. As discussed above, various disease conditions (sepsis, inflammation, obstruction by gallstones) and drugs/hormones effect regulation and function transporters. The mechanisms involved can be divided into (1) transcriptional regulation of genes encoding transporters (this section) and (2) various forms of regulation at the post-transcriptional (protein) level (section 1.6).

1.5.1 Tissue-specific expression of transporters

Tissue-specific expression is controlled by tissue-specific transcription factors. The liver is enriched in Hepatocyte Nuclear Factors (HNF's). Of this family of transcription factors, HNF1α and HNF4 are involved in transcription of hepatic transporters. HNF1α is involved in transcriptional regulation of Ntcp, Oatp1, Oatp2, OATP-C, OATP8, and Oatp4. Another hepatocyte nuclear factor, Hnf4α (NR2A142) is important in hepatocyte differentiation and expression of transcription factors, including Hnf1α, as shown in mice. Hnf4α null mice die during embryogenesis, but a study with conditional Hnf4α-/- mice shows that absence of the hepatic expression of this transcription factor, leads to reduced levels of Ntcp, Oatp1, and Mdr2, while that of Bsep was slightly increased. Thus, hepatocyte nuclear factor 1α and 4α perform important roles in hepatic transporter expression.

The family of CCAAT/enhancer-binding proteins (C/EBP) are other important hepatic transcription factors (for review: 145). C/EBPβ regulates transcription of the MRP2 gene, while an intact C/EBP element is necessary for maximal transactivation of the Ntcp promoter by the transcription factor Rarα. Ntcp gene transcription is also regulated by Hex. Hex is a homeobox-containing protein that is necessary for the normal development of the liver. The Stimulating Protein 1 is an ubiquitously expressed transcription factor. This factor is involved in the hepatic expression of MDR1, Mdr1b, and Mdr2.

1.5.2 Modulation of transcription by NHR's

Nuclear hormone receptors (NHR's) are ligand-activated regulatory proteins and belong to the superfamily of receptors for steroid, retinoids, vitamin D and thyroid hormones (for reviews see: 154,155). This superfamily consists of seven subfamilies, designated 0 to 6. They bind to DNA as monomers, homodimers or heterodimers. Members of this family share a common protein structure, which consists of a ligand-binding domain and a DNA-binding domain. The retinoic X receptor α (RXR, α: NR2B1, β: NR2B2) is the central dimerization partner for many of the family members. The ligands of nuclear hormone receptors are metabolites or drugs that regulate the transcription of genes involved in metabolism and/or transmembrane transport of the ligand itself. The vitamin A derivative, 9-cis retinoic acid is the natural ligand for RXR (NR2B1). The fact that a vitamin A derivative functions as a crucial mediator of gene transcription emphasizes the importance of vitamin A for a wide range of physiological processes.

Several nuclear hormone receptors are involved in transcriptional regulation of hepatic transport proteins. The central factor in regulating bile salt homeostasis is the farnesoid X receptor (FXR; encoded by NR1H4). FXR is expressed in liver, kidney,
Chapter 1: Function and regulation of hepatic transporters involved in bile flow

intestine, and adrenal gland, and is activated by bile salts. Several hepatic transporters are directly regulated by this nuclear receptor together with RXR. Both human and rat BSEP/Bsep expression has been shown to be positively regulated by FXR. Indeed, targeted disruption of Fxr in mice leads to decreased expression of Bsep. Cholate-feeding could not counteract this decrease. The FXR/RXR heterodimer has been described as a permissive. This means that 9-cis retinoic acid activates the dimer and has an additive effect on the activation by bile salts. However, we show in chapter 4 that for BSEP transcription this is not the case. In fact, 9-cis retinoic acid antagonized the effects of bile salts for BSEP transcription. In vivo experiments show that Bsep transcription is especially sensitive to elevated bile salt levels in vitamin A deficient conditions. These findings described in chapter 4 emphasize the important role of vitamin A especially during cholestasis, when vitamin A absorption is compromised.

More and more genes are discovered that are regulated by FXR, including other transporter genes: MRP2, OATP8, and MDR3. FXR also regulates the expression of another nuclear hormone receptor: small heterodimer partner 1 or SHP-1 (NR0B2), which is expressed in liver, small intestine, spleen, adrenal gland, ovary, and testis. SHP-1 is not a typical nuclear hormone receptor since it lacks a DNA-binding domain. SHP-1 performs its function through interaction with other receptors by which it inhibits transactivation mediated by these receptors. For example, Shp-1 inhibits the binding of the Retinoic Acid Receptor (RAR or NR1B1) which is essential for expression of Ntcp. Shp-1 also inhibits the activity of the nuclear receptor liver receptor homologue 1 (LRH-1, NR5A2) and thereby represses the expression of the bile salt synthesis-enzyme cholesterol 7α-hydroxylase. Another target gene for RAR is Mrp2.

The human MRP2 gene promoter is transactivated by three nuclear receptors: besides FXR, these are the Pregnane X Receptor (PXR; NR1I2) and the Constitutive Androstane Receptor (CAR; NR1I3). All three transcription factors activate MRP2 transcription via an everted repeat separated by 8 bases. Thus, the response elements appear to be less factor-specific as previously assumed. Pxr is mainly expressed in liver and intestine, and its ligands are a variety of xenobiotics, but also the more toxic bile salts like lithocholic acid. Pxr also regulates transcription of Oatp2, MDR1, and MRP3. CAR is predominantly expressed in liver and differs from the other nuclear hormone receptors because it transactivates the transcription of target genes in a constitutive manner, i.e. without binding of a ligand, hence the name constitutive androstane receptor. However, a number of CAR activators, including phenobarbital, have been identified to modulate CAR regulation. Therefore, CAR may be considered to be a xenobiotic sensor like PXR. Murine Mdr2 expression is regulated by the peroxisome proliferator-activated receptor alpha (PPARα; NR1C1). It is expressed mainly in liver, kidney and heart, and its ligands include fatty acids and fibrates. PPARα interacts with another nuclear receptor, liver x receptor alpha or LXRα (NR1H3) in a yeast two-hybrid system and inhibits DNA-binding of PPARα/RXRα heterodimers. LXRα is ubiquitously expressed in liver, small intestine, spleen, adrenal gland, ovary, and testis. 

Chapter 1: Function and regulation of hepatic transporters involved in bile flow
expressed\textsuperscript{194} and has been identified as a receptor for the cholesterol metabolites, oxysterols.\textsuperscript{195} Together with the other oxysterol receptor, LXRβ (NR1H2), LXRα transactivates \textit{Abcg5} and \textit{Abcg8} gene expression.\textsuperscript{196}

\subsection*{1.5.3 Regulation of transcription factors during cholestasis}
During cholestasis the protein expression of several hepatic transport proteins is altered. Some of these alterations are caused by down-regulation or up-regulation of the expression of transcription factors. Murine Fxr mRNA and activity is down-regulated upon LPS administration.\textsuperscript{197} For example, Ntcp and Mrp2 are down-regulated during endotoxin-induced cholestasis.\textsuperscript{28,120,124} Studies have shown that cytokines, released in response to endotoxin, suppressed the expression of the proteins\textsuperscript{198} by reducing the formation of RARα/RXRα complexes.\textsuperscript{147} An important survival pathway for cells during cholestasis is represented by Nuclear Factor kappa B (NFκB). This transcription factor is activated by cytokines.\textsuperscript{199} NFκB regulates the transcription of many genes.\textsuperscript{199} These genes encode proteins that are involved in so-called anti-apoptotic pathways. NFκB induction due to inflammatory cholestasis may explain the increased expression of Mdr1 during cholestasis, since NFκB is involved directly in the transcription of \textit{Mdr1b}.\textsuperscript{200} During estrogen-induced cholestasis, down-regulation of the expression of Ntcp and Oatp's may be attributed to a reduced DNA-binding activity of Hnf1, C/EBP and Pxr.\textsuperscript{128}

\subsection*{1.6 Post-translational regulation of transport proteins}
The capacity for transport across the hepatic and intestinal epithelial cells is not only regulated by transcriptional processes, which primarily determine the protein levels. Especially hepatocytes may store significant amounts of transporter proteins in cytoplasmic pools. These cytoplasmic pools may be rapidly targeted to the plasma membrane to increase the transport capacity when needed. This may occur, for instance, immediately after a meal when there is a high demand for bile salt to aid in digestion. Besides acting as FXR-activators, bile salts are also involved in the short-term regulation of the bile salt transport capacity across the canalicular membrane. Taurocholate stimulate bile secretion by translocation of Bsep, but also Mdr1a/b, Mdr2, and Mrp2 from intracellular pools to the canalicular membrane.\textsuperscript{201,202} Similar effects are described for dibutyryl-cyclic AMP, a synthetic second messenger. Cyclic-AMP also stimulates recruitment of Ntcp to the basolateral membrane.\textsuperscript{203} The bile salt tauroursodeoxycholate is used in treatment of certain cholestatic conditions because of its known choleretic actions.\textsuperscript{204} This bile salt activates the PKC and/or MAPK pathways, thereby enhancing the insertion of Bsep\textsuperscript{205} and Mrp2\textsuperscript{206} into the canalicular membrane.

Compounds may also directly inhibit the transport of endogenous substrates, thereby limiting the transport capacity. This is one of the underlying causes of drug-induced (hepato)toxicity. For instance, cholestasis may develop during treatment with cyclosporin A, rifamycin SV, rifampicin or glibenclamide. These drugs have been shown to directly inhibit the transport activity of BSEP.\textsuperscript{207-209} Such drugs may either
selectively inhibit one transporter or have effects on other ABC-transporter family members as well.

1.7 Concluding remarks

Cholestasis has various causes, for instance genetic mutations, sepsis, gallstones or drugs to name a few. But the primary clinical manifestations are the same: jaundice, pruritus, and in the end liver damage. A common cellular phenomenon in cholestatic diseases is dysfunction of transport proteins in bile formation. In the last decade much progress has been made in understanding the function and regulation of hepatic transport proteins. The knowledge gathered gives insight in the mechanism of bile formation in health and disease, and may help to further develop successful therapies for cholestatic diseases.

References

Chapter 1: Function and regulation of hepatic transporters involved in bile flow


Chapter 1: Function and regulation of hepatic transporters involved in bile flow

Chapter 1: Function and regulation of hepatic transporters involved in bile flow


