

Concise Review

Transcriptional regulation of hepatobiliary transport systems in health and disease: Implications for a rationale approach to the treatment of intrahepatic cholestasis

Martin Wagner;¹ Michael Trauner¹

Abstract

Hepatobiliary transport systems mediate hepatic uptake and biliary excretion of bile acids, bilirubin and other biliary constituents. Hereditary or acquired defects of these transporters may cause or maintain cholestasis and jaundice under various clinical conditions including progressive familial intrahepatic cholestasis (PFIC) 1-3 or its milder forms, benign recurrent intrahepatic cholestasis (BRIC) 1 and 2, Dubin-Johnson syndrome, drug and inflammation-induced cholestasis and intrahepatic cholestasis of pregnancy. Moreover, induction of alternative efflux pumps for bile acids/bilirubin and phase I/II detoxifying enzymes may counteract hepatic accumulation of potentially

toxic biliary constituents in cholestasis by providing alternative escape routes. Transcriptional and post-transcriptional regulation of hepatobiliary transporters in health and disease is mediated by multiple factors such as bile acids, proinflammatory cytokines, drugs and hormones. Ligand-activated nuclear receptors (NR) and hepatocyte-enriched transcription factors play a critical role in transcriptional transporter regulation. Many hepatobiliary transporter alterations in cholestatic liver disease can now be explained by ligand binding of accumulating cholephiles to NRs. Moreover, NR-mediated actions may be targeted by pharmacological ligands. Understanding the transcriptional mechanisms leading to transporter changes therefore not only represents a key for understanding the pathophysiology of the cholestatic liver disease, but also represents a prerequisite for designing novel therapeutic strategies.

Key words: Bile formation, bile acids, hereditary and acquired cholestasis, hepatobiliary transporters, hepatocyte-enriched transcription factors, nuclear (orphan) receptors

Introduction

Since the cloning of the major hepatic bile acid uptake system as the first hepatobiliary bile acid transport system in 1991,¹ a substantial amount of further hepatocellular membrane proteins involved in organic anion and bile acid transport together with their substrates have been characterized.²⁻⁴ Ever since numerous hereditary as well as acquired cholestatic liver diseases have been linked to mutated or modified transporter expression.^{2,5} A complex regulatory network at a transcriptional, post-transcriptional, translational and post-translational level mediates the physiological and pathophysiological stimuli resulting in causative or adaptive transporter changes in liver disease.⁶⁻¹¹ While the understanding of the molecular and biochemical mechanisms leading to human liver diseases is becoming more and more detailed an effective treatment is still elusive. This review therefore focuses on recent aspects of transcriptional hepatobiliary transporter regulation under

¹ Laboratory of Experimental and Molecular Hepatology, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Medical University Graz, Austria.

Address for correspondence:
Michael Trauner, M.D.
Laboratory for Experimental and Molecular Hepatology
Division of Gastroenterology and Hepatology
Department of Medicine
Medical University Graz
Auenbruggerplatz 15
A-8036 Graz, Austria
Phone: ++43-316-385-2863, FAX: ++43-316-385-3062
E-mail: michael.trauner@meduni-graz.at

Financial support:

This work was supported by grant P15502 from the Austrian Science Fund, grant 10266 from the Jubilee Funds of the Austrian National Bank and a GEN-AU grant from the Austrian Ministry for Science (to M.T.).

Abbreviations:

ABC, ATP-binding cassette; CAR (NR1I3), constitutive androstane receptor; CBDL, common bile duct ligation; Cyp, cytochrome p450; FXR (NR1H4), farnesoid X receptor/bile acid receptor; HNF, Hepatocyte nuclear factor; Mdr, multidrug resistance gene; Mrp, multidrug resistance-associated protein; Ntcp (Slc10a1), Na⁺/taurocholate cotransporter; Oatp (Slc21a), organic anion transporter; PPAR α (NR1C1), peroxisome proliferator activated receptor α ; PXR (NR1I2), pregnane X receptor; RAR α (NR1B1), retinoic acid receptor α ; RXR α (NR2B1), retinoid X receptor α ; SHP (NR0B2), short heterodimer partner;

physiological and pathophysiological conditions with a special emphasis on regulation by nuclear transcription factors and their implications for future “customized” treatment strategies in cholestatic disorders.

The Enterohepatic circulation

Principles of bile formation

Generation of bile flow is an active process driven by the excretion of organic solutes (bile acids, cholesterol and phospholipids) into the canalicular space between adjacent hepatocytes followed by passive para- and transcellular flux of filterable solutes (e.g. electrolytes, glucose, amino acids) and water. Conjugated bile acids, the major biliary organic solutes are highly concentrated up to 1,000 fold in bile via active transport mechanisms and their vectorial transport from sinusoidal blood to bile represents the major driving force for the so-called bile salt dependent bile flow. Therefore, concentrations of bile acids in the canaliculus and ductules are high (about 20-50 mmol/L) and as high as 300 mmol/L in the gallbladder, while they are diluted to 1–10 mmol/L in the gastrointestinal tract and are as low as 20 – 50 μ mol/L in the portal vein and only 5 μ mol/L after efficient first pass extraction in the systemic venous plasma.¹² Biliary bile acids then induce canalicular phospholipid and cholesterol secretion and form mixed biliary micelles. Bile salt independent bile flow consists mainly of the canalicular secretion of reduced glutathione and the excretion of bicarbonate. The canalicular primary bile is further modified by absorptive and secretory processes along the biliary tree. Considerable species specific differences in bile formation exist including the contribution of ductular bile and bile acid composition.^{2,13-15}

Bile acids undergo an enterohepatic circulation which consists of their continuous hepatocellular canalicular secretion, active re-absorption in the terminal ileum and to lower degrees passive absorption in the colon, and hepatic basolateral re-uptake. Thus, bile acids are getting physiologically accumulated and this bile salt pool in human averages 3-4g and circulates 6-10 times a day. Apart from this major route for bile acids, also cholehepatic shunting (i.e. recirculation of bile acids from the bile duct lumen via cholangiocytes and the peribiliary plexus) and renal-hepatic circulation (i.e. glomerular filtration of bile acids from plasma to urine and reabsorption in the proximal tubule to minimize renal bile acid loss) of bile components occurs, processes which, however gain their full importance mainly under pathological cholestatic conditions.^{2,12} Because of the efficient cycling through liver and intestine as well as minimizing renal loss only 0.5g of bile acids are lost daily by fecal excretion and have to be restored by endogenous conversion of cholesterol to bile acids.

The physiological role of bile comprises digestion and absorption of intestinal lipids and excretion of endogenous (e.g. cholesterol, bile acids, bilirubin) as well as exogenous (e.g. environmental toxins, drugs and their metabolites)

compounds. For a long time the centerpiece in “bile-ology” was thought to be the regulation of cholesterol homeostasis, which is mediated either by biliary secretion of free cholesterol (two thirds) or after conversion of cholesterol into bile acids (one third). However, more and more it turns out that bile acids are not only the waste product of cholesterol, which in turn stimulates bile flow and serves as detergent for dietary fats and vitamins, but have a broad spectrum as signaling molecules with hormone like attitudes. As such bile acids ligand-activate several nuclear receptors involved in bile formation and secretion, detoxification reactions of bile acids and xenobiotics and are even involved in apoptosis or the glucose metabolism.^{2,3,16,17} Thus, it is not surprising that the key step in the formation of bile acid from cholesterol is regulated by complex feedforward and feedback mechanisms.^{11,18}

Basically, bile acids are formed of cholesterol exclusively in pericentral hepatocytes by either the neutral (classic) pathway resulting in the formation of the primary bile acids cholic acid (CA) and chenodeoxycholic acid (CDCA) in roughly equal amounts or the acidic (alternative) pathway, which mainly produces CDCA and may become a more important route in chronic liver disease.¹⁸⁻²¹ Before bile acids are excreted they are conjugated with either glycine or taurine. In the distal intestine the primary bile acids CA and CDCA are deconjugated and deoxygenated by anaerobic bacteria to form the secondary bile acids deoxycholic acid (DCA) and lithocholic acid (LCA). To some extents DCA and LCA are absorbed in the colon and re-conjugated in the hepatocytes. While DCA can constitute up to 20% of total biliary bile acids in man, LCA is additionally sulfated, which limits its intestinal reabsorption. Thus, the highly hepatotoxic LCA constitutes no more than 5% of biliary bile acids.¹² In humans also trace amounts of the 7 β -epimer of CDCA, the therapeutically used ursodeoxycholic acid (UDCA) is formed. In cholestatic liver diseases accumulation of bile acids is counteracted to a certain degree by additional hydroxylation, glucuronidation or sulfation reactions, providing a more hydrophilic water soluble bile acid pool, which in turn can be more efficiently excreted via urine.²²⁻²⁷

Hepatobiliary bile acid transport

Basolateral bile acid uptake and alternative export: Bile acids return to the basolateral (sinusoidal) membrane of hepatocytes mostly in albumin bound form. While dihydroxy bile acids are almost completely albumin bound, protein binding decreases with degree of hydroxylation.¹² These bile acid-albumin complexes pass the fenestrae of the sinusoidal epithelium into the space of Disse, where albumin is dissociated either by the microenvironment of the Disse space or the sinusoidal membrane itself.²⁸⁻³¹ Hepatic first pass effect of conjugated bile acids ranges from 75-90% depending on their structure.²⁸ Under normal conditions this uptake occurs in periportal hepatocytes of zone 1, under cholestatic conditions with higher bile acid concentrations also hepatocytes in zone 3 are recruited.³² Unconjugated

bile acids enter the hepatocyte predominantly by passive diffusion or via Na^+ -independent mechanisms, conjugated bile acids largely by Na^+ -dependent mechanisms. The Na^+ taurocholate co-transporting polypeptide (NTCP) accounts for more than 80% uptake of taurocholate but less than 50% of unconjugated cholate, thus taurocholate is more than 10-fold concentrated in hepatocytes.²⁸ The basolateral Na^+/K^+ -ATPase maintains a transmembranous Na^+ gradient which is the driving force for this Na^+ -dependent transport.⁴ NTCP is exclusively located to the basolateral membrane of hepatocytes throughout the liver and functionally transports all physiological bile acids and to small extents also other compounds³³ (Figure 1, Table I). The bulk of Na^+ -independent bile acid transport is mediated by members of the family of organic anion transporter proteins (OATP/SLC21A – please note the new nomenclature for OATP/Oatp proposed in Hagenbuch et al.),³⁴ which is driven by glutathione and/or bicarbonate anion exchange. In contrast to NTCP, OATPs

are not restricted solely to hepatocytes but are also expressed in multiple tissues including kidney, neuronal structures and intestine. In addition, OATPs also have broader substrate preferences including conjugated and unconjugated bile acids, bilirubin, neutral steroids, eicosanoids, numerous xenobiotics and many more.³⁴ In rodents Oatp1/Slc21a1 (new nomenclature Oatp1a1) mediates most of the bile acid transport, while Oatp2/Slc21a4 (new nomenclature Oatp1a4) and Oatp4/Slc21a10 (new nomenclature Oatp1b2) are of minor importance. In contrast to the homogenous distribution of Oatp1, Oatp2 is predominantly localized to the pericentral region, thus being a potential backup when unextracted bile salts spill over from the periportal region under cholestatic conditions. Among human OATPs, which are not true homologs of the rodent counterparts, OATP2/OATP-C/SLC21A6 (new nomenclature OATP1B1) represents the most important bile salt transporter, while the contribution of OATP-A/SLC21A3 (new nomenclature OATP1A2) is mi-

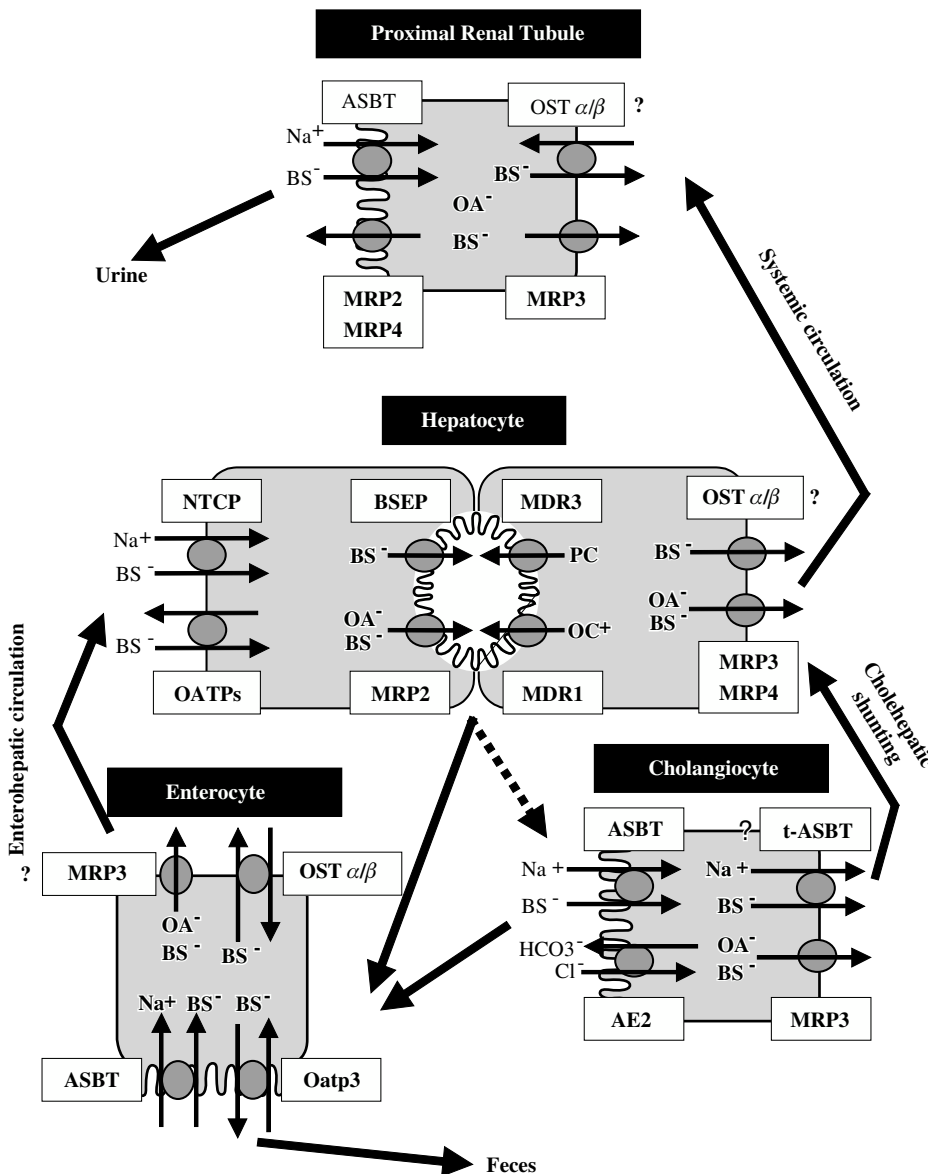


Figure 1. Hepatobiliary transporters in liver and extrahepatic tissues. Bile acids are taken up by the hepatocytes via Na^+ -dependent (NTCP) and Na^+ -independent (OATPs) transport systems. Monovalent bile acids are excreted into the bile canaliculus via BSEP – determining bile salt dependent bile flow-, while divalent bile acids and anionic conjugates (e.g. bilirubin diglucuronide, glutathione) are excreted via MRP2 – determining bile salt independent bile flow. MDR3 mediates the canalicular secretion of phospholipids, which form mixed micelles together with bile acids and cholesterol. MDR1 excretes the bulky of organic cations. Basolateral bile acid export pumps (e.g. MRP3, MRP4) provide an alternative excretory pathway for otherwise accumulated biliary constituents. Bile composition is further modified along the biliary passage by secretion of bicarbonate via AE2 and reabsorption of bile acids via luminal ASBT. A shortcut (called “cholehepatic shunting”) between cholangiocytes and hepatocytes is proposed by basolateral export of bile acids from the cholangiocytes via MRP3 and possibly t-ASBT and hepatocellular reuptake. In the terminal ileum bile acids are reabsorbed by ASBT and to certain degrees in rodents by Oatp3 and effluxed on the basolateral pole of enterocytes via OST α/β and possibly to a lesser extent via MRP3 into portal circulation. Similar to the cholangiocyte and enterocyte, in the proximal renal tubules, bile acids are reabsorbed from the glomerular filtrate via ASBT to minimize bile salt loss. Possibly, renal MRP2 and MRP4 may be involved in the secretion of bile acids into the urine under cholestatic conditions.

Table I. Major hepatobiliary transporters, their function, regulating nuclear receptors, changes in cholestasis and their expected effects in liver and extrahepatic tissue.

Transport protein	Physiological function	Regulatory nuclear receptors and transcription factors	Alterations in cholestatic liver disease	Expected/postulated effects
Basolateral membrane of hepatocytes				
NTCP (SLC10A1)	Na ⁺ -dependent uptake of conjugated bile acids	<i>Positive:</i> RXR:RAR, HNF4, HNF1, HEX, glucocorticoid receptor <i>Repression:</i> SHP, HNF3 β <i>Induction:</i> PXR (Oatp2); FXR (OATP8), RXR:RAR (Oatp4), HNF4, HNF1 <i>Repression:</i> SHP	Reduced	Decreased Na ⁺ -dependent bile acid uptake
OATPs (SLC21A)	Multispecific organic anion transporter for Na ⁺ -independent uptake of bile acid		<i>Reduced:</i> OATP2, Oatp1, rLst1 (liver specific transporter 1, incomplete splicing isoform of Oatp4) <i>Unchanged:</i> Oatp2, Oatp4 <i>Increased:</i> OATP-A	Decreased Na ⁺ -independent bile acid uptake, because most important OATPs for bile acid uptake are affected; maintenance of residual bile acid uptake, putative efflux route by reversal of transport direction
MRP3 (ABCC3)	ATP-dependent export of organic anions including divalent and monovalent bile acid	<i>Induction:</i> PXR, CAR, LRH-1 <i>Repression:</i> RXR:RAR	Increased	Increases alternative bile acid efflux from hepatocyte into circulation
MRP4 (ABCC4)	ATP-dependent export of monovalent and potentially sulfated bile acid in cotransport with glutathione	<i>Induction:</i> CAR	Increased	Increases alternative bile acid efflux from hepatocyte into circulation
OST α /OST β	Na ⁺ -independent transport of monovalent bile acids	Unknown	Unknown	Unknown
Canalicular membrane of hepatocytes				
BSEP (ABCB11)	ATP-dependent export of monovalent bile acids into bile; generating bile acid dependent bile flow	<i>Induction:</i> FXR <i>Repression:</i> HNF4 knockout mice show upregulated Bsep	Mutations cause PFIC2; Initial reduction and later recovery in acquired cholestasis	Hereditary mutations: impaired biliary bile acid excretion Acquired cholestasis: continuation of bile acid export and maintenance of bile salt dependent bile flow
MRP2 (ABCC2)	ATP-dependent export of divalent but not monovalent bile acids and bilirubin diglucuronide into bile; contributing to bile salt independent bile flow via GSH excretion	<i>Induction:</i> FXR, PXR, CAR, RXR:RAR	Mutations cause Dubin-Johnson Syndrome; Reduced in acquired cholestasis	Impairment of biliary excretion of amphiphilic compounds, including bilirubin, drugs, bile acids; Reduction of bile acid independent fraction of bile flow
MDR1 (ABCB1)	ATP-dependent export of organic cations into bile	<i>Induction:</i> PXR	Increased	Increased efflux of xenobiotics and other yet unidentified cholephiles
MDR3 (ABCB4)	ATP-dependent translocation of phosphatidylcholine from the inner to the outer leaflet of the canalicular membrane; generating phospholipid secretion	<i>Induction:</i> PPAR α , Mdr2 is downregulated in conditional HNF4 knockout mice <i>Repression:</i> HNF3 β	Mutations cause PFIC3 and are associated with Intrahepatic cholestasis of pregnancy; Unchanged in acquired cholestasis	May affect critical micellar biliary concentration of bile acids by phospholipid secretion
FIC1 (ATP8B1)	ATP-dependent putative aminophospholipid translocase	<i>Repression:</i> SHP	Mutations cause PFIC1; Unchanged in acquired cholestasis	Unknown
AE2 (SLC4A2)	HCO ₃ ⁻ secretion into bile; contribution to bile acid independent bile flow	Potentially glucocorticoid receptor	Reduced	Reduced HCO ₃ ⁻ secretion into bile
Cholangiocytes				
ASBT (SLC10A2)	Apical localization; Na ⁺ -dependent uptake of monovalent bile acids from the bile	<i>Induction:</i> PPAR α , glucocorticoid receptor, in HNF1 knockout mice intestinal Asbt is reduced, activating protein-1 (AP-1), LRH-1, RXR:RAR	Increased	Increased biliary reabsorption of bile acids

MRP3 (ABCC3)	Basolateral localization; may be involved in the return of bile acids from bile to portal circulation	<i>Induction:</i> PXR, CAR, LRH-1 <i>Repression:</i> RXR:RAR	Unchanged	May mediate efflux of bile acids via basolateral membrane into the peribiliary capillary plexus back to the liver
AE2 (SLC4A2)	Apical localization; HCO ₃ ⁻ secretion into bile; contribution to bile acid independent bile flow	Potentially glucocorticoid receptor	Reduced in PBC and unchanged in ethinyl estradiol induced cholestasis in rodents	Reduced HCO ₃ ⁻ secretion into bile
Intestine				
ASBT (SLC10A2)	Apical localization explicitly in the ileum; Na ⁺ -dependent uptake of monovalent bile acids from the intestine; critically in enterohepatic circulation	<i>Induction:</i> PPAR α , glucocorticoid receptor, HNF1 (in knockout mice), activating protein-1 (AP-1), LRH-1, RXR:RAR	Reduced	Reduced intestinal reuptake of bile acids and subsequently enhanced fecal excretion
MRP3 (ABCC3)	Basolateral localization in duodenum, ileum, caecum and colon; contribution to the return of bile acids into portal circulation unclear	<i>Induction:</i> PXR, CAR, LRH-1 <i>Repression:</i> RXR:RAR	Potentially induced	Potential effect on reabsorption of bile acids from intestine to portal circulation
OST α /OST β	Basolateral localization to highest extent in ileum; may be the major transporter for the return of bile acids into portal circulation	Unknown	Unknown	Potential effect on reabsorption of bile acids from intestine to portal circulation
Oatp3	Apical localization along the intestine; Na ⁺ -independent uptake of bile acid from intestinal lumen	Unknown	Unknown	Potential effect on reabsorption of bile acids from intestine to portal circulation
Kidney				
ASBT (SLC10A2)	Apical localization; Na ⁺ -dependent uptake of monovalent bile acids from the glomerular filtrate; critically in renal-hepatic circulation	<i>Induction:</i> PPAR α , glucocorticoid receptor, HNF1 (in knockout mice), activating protein-1 (AP-1), LRH-1, RXR:RAR	Reduced	Reduced reabsorption of glomerular filtrated bile acids and subsequent enhanced renal excretion
MRP2 (ABCC2)	Apical localization; ATP-dependent transport of divalent bile acids; postulated to contribute to active renal bile acid excretion	<i>Induction:</i> FXR, PXR, CAR, RXR:RAR	Induced (rats), Unchanged (mice)	Induced active renal excretion of bile acids
MRP4 (ABCC4)	Basolateral localization; ATP-dependent transport of monovalent and potentially sulfated bile acid in cotransport with glutathione; postulated to contribute to active renal bile acid excretion	<i>Induction:</i> CAR	Induced (mice), Reduced (rats)	Induced active renal excretion of bile acids
OST α /OST β	Na ⁺ -independent transport of bile acid; role in kidney undetermined	Unknown	Unknown	Unknown

nor and OATP-B/SLC21A9 (new nomenclature OATP2B1) does not transport bile acids. The role of OATP-8/SLC21A8 (new nomenclature OATP1B3), a gene duplication variant of OATP2, in bile acid transport is still controversial.^{2,4,13} In addition, organic anion transporters (Oat), localized to the basolateral membrane of kidneys and one isoform (Oat2) also to hepatocytes may additionally modulate hepatic drug and bile acid uptake, although specific bile acid transport has so far only been shown for the renal isoforms.³⁵

Retrograde hepatocellular bile acid efflux via the basolateral membrane may become an important alternative

spill over route for accumulating bile acids/bilirubin during cholestatic disorders. The most important adaptive transport systems ruled out so far belong to the class of multidrug resistance ATP-binding cassette (ABC) transporters (MRPs). Mrp1/Abcc1/MRP1/ABCC1, Mrp3/Abcc3/MRP3/ABCC3 and Mrp2/Abcc4/MRP4/ABCC4 are constitutively expressed only at very low levels at the basolateral hepatocyte membrane, but are inducible under cholestatic conditions. Their substrate specificity comprises divalent bile acids and in the case of MRP3 and MRP4 also monovalent bile acids.^{2,35-38} Also MRP5/ABCC5 and

MRP6/ABCC6 are located to the basolateral membrane of hepatocytes, however, their role in adaptive bile salt transport remains to be further explored.

Under bile acid burden also members of the Oatp family remain candidates for supporting bile acid efflux, since Oatp1 and Oatp2 were shown to act as bidirectional exchangers in *Xenopus laevis*.³⁹ The new kid in bile acid transport is the heteromeric organic solute transporter OST α and OST β which was recently shown to transport taurocholate in a Na⁺-independent manner. However, only the human isoforms were shown to be expressed in hepatocytes as well.⁴⁰ The murine isoforms were abundantly expressed in small intestine and to lesser extents in kidney, where their major function is addressed to be the basolateral counterpart of apical Asbt.⁴¹

Canalicular bile acid excretion: Once being taken up at the basolateral membrane, bile acids are transferred to the canalicular membrane for rapid excretion.⁴ Under physiological conditions hepatocellular bile acid binding proteins, so far 3 α -hydroxysteroid dehydrogenase (3 α -HSD), glutathione S transferases (Gst) and the liver fatty acid binding proteins (L-FABP), are involved in this intracellular transfer.⁴² Under conditions of high bile acid load hydrophobic bile acids may also be transferred within membrane-bound compartments.^{2,4} In bile formation canalicular bile acid excretion represents the rate limiting step. Similar to the basolateral membrane the most important export pumps for canalicular bile acid excretion belong to the class of ATP-dependent ABC transporter systems. Under normal conditions the bile salt export pump, Bsep/Abcb11/BSEP/ABCB11, represents the major bile acid transport systems for monovalent bile acids, actively transporting its substrates against a 1,000-fold concentration gradient. While the loss of BSEP in humans, causing hereditary progressive familial intrahepatic cholestasis type 2 (PFIC2), results in a decrease of biliary bile acid concentration to 1% of the normal range,⁴³ the knock-out of Bsep/Abcb11 in mice only results in a decrease of 30% percent⁴⁴ suggesting the presence of other canalicular bile acid transport systems. Although these additional transport systems have not been identified so far, Mrp2/Abcc2 is a candidate for transporting divalent, glucuronidated and sulfated⁴⁵ and also tetrahydroxylated bile acids.² In addition, bilirubin, GSH and a broad range of amphipathic compounds are excreted by Mrp2 as well, however, monovalent bile acids are no substrates for Mrp2.⁴⁶ An additional transport systems for sulfated conjugates, including dehydro-epiandrosterone sulfate and estrone-3 sulfate is the breast cancer related protein (Bcrp/Abcg2).⁴⁷ After secretion biliary bile acids drive the secretion of phosphatidylcholine and cholesterol from the outer leaflet of the canalicular membrane. The continuous support of phospholipids from the inner to the outer leaflet is mediated by the phospholipid flippase Mdr2/Abcb4 or its human ortholog MDR3/ABCB4.⁴⁸ The canalicular transport of cholesterol is most likely mediated by the sterol half-transporters Abcg5/8.⁴⁹ Together bile acids, phospholipids and cholesterol form mixed micelles which in

turn avoid bile acid toxicity to the bile duct epithelium.² Additional canalicular transporters include the multidrug export pump MDR1/ABCB1 (in rodents Mdr1a/Mdr1b) which accounts for the transport of bulky amphipathic cations (e.g. drugs) and the putative aminophospholipid transporter FIC-1/ATP8B1, which mutations causes familial intrahepatic cholestasis type 1.² The most relevant ATP-independent transporter located to the canalicular membrane is a Cl⁻/HCO₃⁻ exchanger (AE2/SLC4A2) which together with MRP2 accounts for the greatest part of the canalicular bile acid independent bile fraction.⁵⁰

Cholangiocellular bile acid transport

Cholangiocytes are important modulators of canalicular bile by secreting and reabsorbing processes while bile passes the intrahepatic biliary tree. Experiments in rats suggest that only large bile ducts (>15 μ m diameter) contribute to these changes while small bile ducts remain passive.⁵¹ While the small amount of monomeric unconjugated bile acids may passively enter cholangiocytes in exchange for HCO₃⁻, thus inducing bicarbonate rich hypercholerisis, a mechanism called cholehepatic shunting and inducible by dihydroxy bile acids and C-23 Nor-dihydroxy bile acids,^{4,52} the majority of biliary bile acids is conjugated and can be reabsorbed in a Na⁺-dependent manner via the apical bile acid transporter Asbt.⁵³ After uptake and potential conjugation during transcellular transport bile acids may be effluxed to the plasma of the periductular capillary plexus via an anion exchange mechanism.⁵⁴ Candidates for this mechanism are the human OATP-A⁵⁵ or the rodent Oatp3/Slc21a7 (new nomenclature Oatp1a5).¹³ In addition, this could also be achieved by Mrp3/MRP3, which is located to the basolateral membrane of cholangiocytes^{56,57} or a splicing variant of Asbt/SLC10A2 so called truncated Asbt (t-Asbt).⁵⁸ Similar to the function of Ost α /Ost β in the intestine⁴¹ it could well be imagined that this heterodimeric transport systems functions also in the cholangiocytes as counterpart of Asbt. To complete, also the Cl⁻/HCO₃⁻ anion exchanger AE2 and cystic fibrosis transmembrane regulator CFTR/MRP7/ABCC7 both most important for ductal bile secretion are expressed only in large bile ducts.⁵¹

Intestinal bile acid transport

To complete enterohepatic circulation an efficient reabsorption of bile acids in the intestine with subsequent delivery to portal circulation is required. Again, while parts of unconjugated bile acids may be absorbed by passive diffusion, conjugated bile acids are taken up into enterocytes by Na⁺-dependent and Na⁺-independent mechanisms.⁴ The first one is mediated by the apical sodium-dependent bile salt transporter Asbt, which is similar to the one also found in cholangiocytes and renal tubular cells.⁵⁹ Asbt is strictly restricted to the terminal ileum,

where it solely mediates the transport of bile acids, conjugated and unconjugated primary and secondary bile acids.⁵⁹ Na⁺-independent bile acid transport for conjugated and unconjugated bile acids is mediated by Oatp3, which in contrast to Asbt is expressed down the length of the small intestine.⁶⁰ If this holds true for human OATP expression (i.e. OATP-A) in intestinal brush border membranes remains to be further evaluated.^{4,61} Intracellular trafficking of bile acids is mediated via the intestinal bile acid-binding protein, I-Bapb, which is cytoplasmatically attached to Asbt.⁶² For long it was speculated that either t-Asbt⁵⁸ or Mrp3^{63,64} may represent the pendant to Asbt for basolateral bile acid export. However, recent findings suggest that heteromeric Ost α /Ost β , which catalyzes Na⁺-independent bile acid export only in its heteromeric form^{40,41} is the more likely candidate since it exactly mirrors Asbt distribution in intestine and kidney.⁴¹ One has to mention that also export pumps are expressed in apical brush border membranes (e.g. Mdr1, Mrp2).² Concerning bile acid transport, Mrp2, which is highest expressed in duodenum and continuously decreases towards colon,^{65,66} might play a role as alternative defense mechanism.⁶⁷

Renal bile acid transport

Under normal conditions, bile acids which escape first pass effect of the liver are glomerulantly filtered and effectively reabsorbed in the proximale nephron convolute.⁶⁸ In cholestasis however, this pathway can gain more importance by reduced re-uptake and enhanced active secretion.⁶⁹ Similar to the terminal ileum re-uptake is mediated by the apical located Asbt. While unconjugated and conjugated di- and trihydroxy bile acids can be transported via this salvage mechanism, sulphated bile acids are not transported, thus promoting the excretion of these compounds which are highly formed in cholestatic conditions.³⁵ Also Oatp1 has been located to the apical membrane of the proximale tubules,⁷⁰ thus potentially contributing to bile acid re-absorption. The role of Oatp3 in the kidney is still controversial.³⁵ The contribution of active urinary bile acid secretion via apical Mrp2 and Mrp4, which are upregulated in cholestatic conditions^{67,71,72} has been postulated, but so far not been proven in detail. In analogy to rodents also the human counterparts have been localized in the kidney, i.e. ASBT, OATP-A, MRP2 and MRP4.^{59,73-75} Little is known about basolateral efflux of bile acids in the kidney. Potential candidates are Mrp3,^{72,76} organic ion transporters (Oat),³⁵ or again the counterpart of Asbt on the basolateral membrane Ost α /Ost β , which is highly expressed in the kidney.^{40,41}

Transcriptional regulation under physiologic conditions

The regulation of bile acid transport systems is subject to intensive control mechanisms on transcriptional and post-transcriptional levels. On transcriptional level a

complex interacting network of ligand activated nuclear (orphan) receptors (NR) and hepatic transcription factors catalyzes the physiological and pathophysiological demands. NR involved in the regulation of hepatobiliary transport systems are either directly ligand activated by distinct bile acids, steroid hormones, retinoids and a broad spectrum of diverse drugs or their nuclear availability and DNA binding can be modified (mainly by cytokines).^{8,9,11,77} Another level in mediating nuclear receptor function comprises recruitment of distinct co-activators and release of co-repressors.⁷⁸ So far, three nuclear receptors, the farnesoid X receptor FXR (NR1H4), the pregnane X receptor PXR (NR1I2) and the vitamin D receptor VDR (NR1I1), have been shown to bind a distinct spectrum of hydrophobic bile acids as ligands.⁷⁹⁻⁸⁴ In addition, the liver X receptor alpha LXR α (NR1H3) was demonstrated to be activated by 6 α -hydroxy bile acids and analogs.⁸⁵ The constitutive androstane receptor CAR (NR1I3) is endogenously activated by bilirubin⁸⁶ but a role for bile acid sensing has been postulated.⁸⁷ All of these NR belong to class II nuclear receptors, that in contrast to class I steroid hormone receptors typically form heterodimers with the retinoid X receptor alpha RXR α (NR2B1) before binding to distinct DNA response elements in respective promoter regions. It is noteworthy that RXR can function as a silent heterodimer partner (e.g. for VDR, RAR) – RXR ligand binding is prevented – or as an active heterodimer partner (e.g. for FXR, LXR, PPAR) – both receptors can be synergistically activated/modulated in the presence of both ligands.^{88,89} Moreover, bile acids and cytokines are able to directly affect expression levels and activity of critical hepatic transcription factors, which are essentially for hepatobiliary transporter expression.^{2,90-92} Rapid changes of transporter expression are mainly mediated by posttranscriptional actions. An intensive regulation by second messengers and protein kinases modulates recruitment of transporters from intracellular pools to the plasma membrane and alters carrier activity by (de)/phosphorylation and protein-protein interaction processes.^{2,3,7} However, this review will keep its focus on transcriptional regulation and for a more detailed understanding the reader is kindly referred to recent more detailed reviews.^{2,6,7}

FXR: The farnesoid X receptor (FXR/NR1H4) was the first and most important nuclear orphan receptor shown to be activated by bile acids.⁷⁹⁻⁸¹ FXR is abundantly expressed in tissues belonging to the enterohepatic circulation (i.e. liver, gut) and in the kidney.⁹³ More recent investigations found FXR also expressed in several tissues not belonging to typical bile acid target tissues (e.g. heart, thymus, ovary, eye, spleen, testis, vasculature and vascular smooth muscle cells).⁹⁴⁻⁹⁶ Physiological bile acids were reported to activate FXR in the rank order of potency: CDCA (chenodeoxycholic acid) > DCA (deoxycholic acid) = LCA (lithocholic acid) > CA (cholic acid).⁷⁹ However, recent data suggest that LCA is an inverse agonist of FXR⁹⁷ and DCA, CA and UDCA are

partial FXR agonists which regulate FXR target genes in a gene-selective manner.⁹⁸ Smiliar or even higher FXR binding and activity was shown for the conjugated bile acids.^{79,99} FXR typically forms a heterodimer with RXR and binds with high affinity to inverted repeat (IR) 1 response elements, although binding to IR0 (for SULT2A1) and everted repeats (ER) 8 (for MRP2) where also shown.¹⁰⁰⁻¹⁰³ One prominent exception from this rule is human UGT2B4 - glucuronidating hydrophobic bile acids - which promotor binds FXR as a monomer.¹⁰⁴ Since bile acids represent the natural ligands for FXR activation it is not surprising that this receptor activates and represses key transporters of bile acid uptake and export as well as key enzymes of bile acid synthesis and metabolism via direct or indirect mechanisms. Both canalicular bile acid export transporter genes BSEP/Bsep and MRP2/Mrp2 are directly transactivated via FXR:RXR binding to IR-1 or ER-8 response elements in their promotor regions, respectively.^{102,105-107} It is noteworthy that LCA may act as an FXR antagonist thus downregulating (stimulated) Bsep expression.^{97,108} While none of rodent Oatps is reported to be regulated via FXR, human OATP1B3/SLC21A8 (formerly OATP8) is directly transactivated via the IR-1 response element¹⁰⁹ and human OATP-C is repressed (see below). Also MDR3 belong to the genes which are directly positively regulated by FXR.¹¹⁰ Apart from bile acid and organic anion transporters also human and murine ileal bile acid binding protein (I-BABP/I-babp) were shown to be directly transactivated,^{80,111,112} further the rat bile acid bile acid-CoA: amino acid N-acetyltransferase (BAT), which taurine and/or glycine conjugates bile acids¹¹³ and bile acid sulfating sulfotransferase (STD or Sult2a1).^{103,114} The regulation of FXR itself in cholestasis is controversial. While there are reports that FXR is downregulated after common bile duct ligation in rats¹¹⁵ and CA feeding in mice,¹¹⁶ (Trauner & Wagner, unpublished observations), recent reports rather suggest a positive auto-regulatory loop for FXR.^{98,117} Repressive effects of FXR on hepatobiliary transporters are mediated indirectly via the short heterodimer partner (SHP) and/or hepatocyte nuclear factors (HNF) 4 and 1 involving bile acids as well as cytokines. The promotor of human and rodent SHP, contains FXR response elements and is positively transactivated by FXR.¹¹⁸ However, a negative feedback loop for this transactivation via SHP inhibition of the FXR:RXR coactivator PGC-1 seems to fine tune this mechanism.¹¹⁹ SHP itself, highly expressed in liver, is an unusual NR since it lacks the DNA binding domain but still contains its dimerization domain allowing to interact and negatively affect several other NR (among them HNF4, liver receptor homologue-1 (LRH-1), CAR:RXR, RAR:RXR, PXR:RXR, LXR:RXR) but positively affects PPAR α :RXR and PPAR γ :RXR.⁸ SHP mediated repression is suggested to involve competition with coactivators and its strong transcriptional repressor domain.^{8,120,121} So far no ligand has been reported for SHP. FXR-SHP dependent repression has been reported for rat Ntcp via repression of HNF4 and RAR:RXR binding^{120,122} and suggested for mu-

rine Ntcp.¹²³ However, Ntcp downregulation upon bile acid feeding in SHP knock-out mice also seem to involve bile acid dependent, but SHP independent pathways.¹²⁴ Such pathways might involve bile acid (\pm cytokine induction) triggered activation of the JNK pathway and reduced binding of HNF4 (proposed for rat Cyp7a1),^{125,126} JNK dependent phosphorylation of RXR with reduced binding of RAR:RXR to the rat Ntcp promotor,¹²¹ cytokine induction with suppression of RAR:RXR, HNF1 and HNF4 (shown for rat Ntcp promotor),^{90,127,128} bile acid \pm cytokine mediated reduced HNF4 binding (proposed for rat Ntcp and human OATP1B1 (formerly OATP-C)).^{91,129,130} However, concerning transcriptional Ntcp regulation there might exist great species specific differences.⁹¹ It should be mentioned at this point that HNF1 is negatively regulated by HNF4 and HNF1 in turn negatively regulates its own expression and that of other HNF4 dependent genes.¹³¹ The central role of HNF1 and HNF4 as central regulators of basolateral bile acid uptake transporters is underlined by reduced Ntcp, Oatp1 and Oatp2 expression in HNF1^{-/-} and conditional HNF4^{-/-} mice.^{132,133} HNF1 α was also reported to be required for expression of Oatp4.^{134,135} Thus, HNF1 α appears to be the master regulator of basolateral Ntcp and Oatp expression. Among hepatobiliary transporters human OATP1B1 is also negatively regulated by FXR involving HNF1 and HNF4.^{8,91} Mouse Asbt is downregulated by bile acids involving FXR-SHP dependent reduced LRH-1 binding to the Asbt promotor,¹³⁶ while human ASBT promotor is suggested to be negatively regulated via SHP dependent reduced RAR:RXR transactivation.¹³⁷ The HNF1 knockout mouse also represents reduced Asbt.¹³² The transcriptional effects of FXR-SHP dependent and independent pathways on the regulation of CYP7A1, CYP8B1 and CYP27, involving LRH-1 and again as central regulator HNF4 should only be mentioned and the reader is referred to detailed recent reviews.^{2,8,18}

PXR: The pregnane X receptor (PXR/NR112 or SXR in human) was the second NR reported to be activated by bile acids in the order of potency: 3-keto LCA, LCA, and to lesser extents by DCA and CA.^{82,83} One report also suggested UDCA as a potential ligand of PXR.¹³⁸ Because of a variable ligand binding domains (LBD) there exist great differences in distinct PXR ligands between species.^{139,140} As such, rifampicin, which is a known human PXR activator do not activate rodent PXR while pregnenolone-16 α carbonitrile (PCN) acts vice versa.^{141,142} Also PXR ligands itself comprise a heterogenous group including bile acids, vitamin E,¹⁴³ a spectrum of several xenobiotics, including rifampicin, natural steroids or hyperforin, the active compound of St. John's wort.^{141,142,144} PXR binds as PXR:RXR heterodimer to direct, indirect and everted repeats spaced by a various number of nucleotides⁹ and it is highly expressed in liver and intestine and to lower degrees in kidney, uterus, ovary and placenta.^{9,141,145} Although its primary role is determined to be xenobiotic detoxification through transactivation of human and rodent cyto-

chrome p450 CYP3A and CYP2B isoforms⁷⁷ as well as activation of the human MDR1,^{146,147} several recent reports recognize its capability in modulating hepatobiliary transport systems and bile acid metabolizing enzymes (for an profiling of PXR target genes see¹⁴⁸). So far rodent Oatp2 has been shown to be PXR dependent⁸³ and there are also data for a positive regulating role in the transcription of MRP3/Mrp3.^{149,150} Together with FXR and CAR, PXR also regulates MRP2/Mrp2 expression via an common unusual ER8 element in the promotor.¹⁰² Similar to FXR, PXR represses CYP7A1 and potentially CYP8B1 via a mechanism again involving HNF4.^{151,152} In addition, Sult2a1^{114,153} and bilirubin glucuronidating Ugt1a1^{154,155} are PXR dependent. A recent report also found a PXR binding site in the human SHP promotor¹⁵⁶ and in turn human and murine PXR is negatively regulated by SHP.¹⁵⁷

CAR: The constitutive androstane receptor CAR (NR1I3) has so far not been shown to bind bile acids, but to be activated by bilirubin, which accumulates in cholestatic disorders.⁸⁶ Similar to PXR there is also a significant ligand dependent species variability.¹⁴² Androstane metabolites are reported to be inverse agonists, while phenobarbital is a well known CAR agonist.¹⁵⁸ CAR again binds as CAR:RXR heterodimer to several DR and ER motifs,⁹ but there has also been described a binding of CAR as monomer.¹⁵⁹ Unlike other NRs CAR is constitutively active meaning that it do not require an agonist for full activation and it is sequestered in the cytoplasm from where it translocates to the nucleus similar to classic steroid hormone receptors.^{8,9,58} CAR is highly expressed in the liver and intestine.^{160,161} CAR and PXR regulate distinct but overlapping genes involved in bilirubin, bile acid and xenobiotic metabolism,¹⁵⁵ part of this action may be mediated by binding of CAR and PXR to common response elements in promotor regions of CYP2B and CYP3A genes.^{142,162} So far CAR is shown to participate in the regulation of several Mrps, including Mrp2,¹⁰² Mrp3,^{65,163} Mrp4,^{87,150} Oatp2^{116,150} and bile acid and bilirubin detoxification enzymes such as Sult2a^{87,114,150,164} and Ugt1a1.^{86,165} Again, SHP may interact indirectly with CAR via recruitment of co-repressors or inhibition of co-activators and is thus able to inhibit the transactivation of the classical CAR target gene CYP2B.¹⁶⁶

Other nuclear receptors involved in hepatobiliary transporter regulation (VDR, RAR, LRH-1, PPAR α , GR): Recently also the vitamin D receptor VDR (NR1I1) was shown to be activated by LCA and its metabolites in even lower concentrations than required for PXR activation.⁸⁴ The expression of VDR in hepatocytes is minimal and significantly higher in Kupffer and stellate cells and the intestine.¹⁶⁷ While so far only bile acid detoxifying enzymes (i.e. Cyp3A4/Cyp3a11, Sult2a1) have been shown to be regulated by VDR,^{84,114} a most recent report identified a VDR response element in the rat Asbt gene and its induction by calcitriol,¹⁶⁸ suggesting that VDR may even play a role in bile acid transporter regulation. Moreover,

FXR induced BSEP expression was also inhibited by vitamin D.¹⁶⁹ The retinoic acid receptor RAR α (NR1B1), the most prevalent isoform in hepatocytes is activated by retinoids.⁹ The obligate RAR:RXR heterodimers is required for constitutive expression of rat Ntcp.¹²⁸ Moreover, RAR:RXR binding to retinoid response elements in the promotor regions of Ntcp and Mrp2 positively transactivates both genes,¹²⁸ a similar mechanism is suggested for Oatp4.¹³⁵ In contrast, RAR α :RXR α is suggested to negatively regulate Mrp3.¹⁷⁰ Liver receptor homologue LRH1 (NR5A2) (also known as fetoprotein transcription factor, FTF) is involved in the upregulation of Mrp3, since a binding site for LRH-1 was found in the Mrp3 promotor.¹⁷¹ Since bile acids do not act as LRH-1 ligands¹⁷² another factor mediating bile acid-dependent induction might be required.¹⁷¹ TNF α may be this factor since it up-regulates LRH-1 expression *in vitro* and *in vivo*.¹⁷³ Involvement of LRH-1 has also been suggested for Asbt regulation² and CYP8B1.¹⁷⁴ Peroxisome proliferating activator receptor PPAR α (NR1C1), activated by fibrates, statins, long chain unsaturated fatty acids and eicosanoids is involved among others (for review)⁹ in the regulation of MDR2,¹⁷⁵ ASBT,¹⁷⁶ CYP8B1¹⁷⁷ and CYP7A1,¹⁷⁸ UGT2B4¹⁷⁹ and SULT2A1.¹⁸⁰ The glucocorticoid receptor GR (NR3C1) is shown to directly transactivate human NTCP^{8,181} and human ASBT¹⁷⁶ and a role in the regulation of AE2 was discussed.¹⁸² Recently and GR response element was found in the human CAR promotor.¹⁸³ Most interesting, UDCA was reported to activate the glucocorticoid receptor.¹⁸⁴

Changes in disease

Impairment of normal bile flow leading to cholestasis may be the result of either functional defects in bile formation at the hepatocellular level or in secretion and flow at the bile duct level.^{6,185} Transporter alterations play an important role in the development and/or maintenance of these cholestatic disorders, but in turn may also provide anticholestatic defense mechanisms. In general, downregulation of basolateral Ntcp and Oatps, together with partially recovery of Bsep reduces hepatocellular accumulation of potentially toxic bile acids. In addition, induction of basolateral Mrp3 and Mrp4 may provide an alternative pathway for elimination of biliary compounds. Moreover, cholestasis leads to an impairment of the normal enterohepatic circulation with enhanced ductular re-uptake of bile acids via induction of cholangiocellular Asbt ("cholehepatic shunting") and – reciprocally – reduced renal re-uptake of biliary compounds via downregulated renal Asbt, thus together with induction of renal Mrp 4 promoting bile acid elimination.^{2,186} Therefore, defects in transporters may be primary as the result of genetic defects or secondary acquired as a result of cholestatic injury (most forms of transporter alterations). Several animal models mimicking human intrahepatic and extrahepatic cholestasis have enabled

us to understand the transporter alterations and pathophysiological mechanisms on which these diseases are based. In the following, genetic and acquired human cholestatic liver disease with their corresponding animal model (mainly results from mice and rats) are confronted and general aspects are highlighted.

Genetic transporter alterations

Progressive familial intrahepatic cholestasis 1 (PFIC1): PFIC1 (Byler's disease) often begins first as recurrent and later permanent cholestasis leading to fibrosis, cirrhosis and liver failure in the first 2 decades of childhood. Typically, children represent with low γ -glutamyltransferase levels and high primary bile acids in serum as well as extrahepatic manifestations (e.g. diarrhea, malabsorption and pancreatitis, hearing impairment). Histologically, a bland canalicular cholestasis without much bile duct proliferations impresses and electronmicroscopically the bile appears coarse granular in the canaliculi (Byler bile).^{5,187,188} PFIC1 is caused by mutations in FIC1 (ATP8B1), an aminophospholipid flippase which is expressed in the canalicular membrane of hepatocytes and cholangiocytes.^{189,190} In contrast to previous suggestions^{191,192} FIC1 appears not to be a bile acid transporter. Thus the mechanisms, by which mutations in this gene cause a profound decrease in biliary (hydrophobic) bile acids, remains unresolved.¹⁹³ Of interest, symptoms of diarrhea persist after liver transplantation, suggesting a broader role of FIC1 in disease development.² FIC1 knock-out mice show high intestinal bile acid uptake upon bile acid feeding suggesting a defect in the regulation of intestinal bile acid absorption.¹⁹⁴ A recent study found FXR downregulated and reduced in its activity in some PFIC1 patients which was accompanied by increased expression of ileal ASBT and reduced expression of ileal bile acid binding protein (ILBP).¹¹⁷ Similar to FIC1 knock-out mice, the amount of reabsorbed bile acids in FXR knockout mice was increased, despite absence of ileal bile acid binding protein,¹⁹⁵ thus giving a rationale for the intestinal findings in patients and mice. Moreover, reduced hepatic BSEP expression secondary to FXR alterations might account for some of the hepatic changes in these patients, although this hypothesis needs to be proven.¹¹⁷ Mutations in the FIC1 gene are also associated with the benign recurrent intrahepatic cholestasis (BRIC type 1, Summerskil Syndrome), which represents with recurrent episodes of cholestasis and low γ -glutamyltransferase levels and can also be associated with extrahepatic symptoms, but does not progress to chronic liver disease.^{5,191}

Progressive familial intrahepatic cholestasis 2 (PFIC2): PFIC2 (Byler's syndrome) also do not show elevated levels of γ -glutamyltransferase or bile duct proliferation but histology often presents with nonspecific giant cell hepatitis and patients are frequently jaundiced and the disease rapidly progresses to progressive cholestasis requiring liver transplantation within the first decade. In

contrast to PFIC1 the bile is amorphous or filamentous and extrahepatic manifestations are uncommon⁵ (with the exception of cholelithiasis, which is found in PFIC2/BRIC2, but not in PFIC1/BRIC1).¹⁹⁶ PFIC2 is caused by mutations in BSEP,¹⁹⁷ thus patients only have 1% of normal biliary bile acid secretion, confirming the predominant role of this transporter for human canalicular bile acid secretion.⁴³ Surprisingly, Bsep knockout mice only display mild nonprogressive cholestasis.⁴⁴ This less severe phenotype in mice can likely be attributed to another yet unidentified canalicular bile acid transport system, which in contrast to Bsep may transport mainly hydrophilic bile acids, which are more prominent in rodents than in humans.^{44,198} Similar to mutations in FIC1 less severe missense mutations in the BSEP gene may also cause a form of benign recurrent intrahepatic cholestasis (BRIC type 2).¹⁹⁶

Progressive familial intrahepatic cholestasis 3 (PFIC3): PFIC3 is caused by homozygous mutations in MDR3.¹⁹⁹ In contrast to PFIC1 and PFIC2, PFIC3 is characterized by high γ GT values, bile duct proliferation and inflammatory infiltrates in portal fields.²⁰⁰ When the phospholipid flippase is deficient, biliary phospholipid content dramatically decreases, thus bile acids do not form mixed micelles and the lack of their protective effects on the bile duct epithelium may cause bile acid-induced injury of the biliary epithelium.¹⁹⁹ The spectrum of MDR3 deficiency is broad ranging from neonatal cholestasis and intrahepatic cholestasis of pregnancy (ICP) to cirrhosis in adults and cholesterol gallstone formation.^{55,201,202} Actually, in some cases it may become difficult to distinguish between fully reversible ICP with MDR3 mutations from more serious MDR3 associated liver disease.^{186,203} The pathophysiological consequences of this genetic defect were established in the Mdr2 knockout mouse,⁴⁸ which completely lacks biliary phospholipid levels and develop a phenotype resembling primary sclerosing cholangitis²⁰⁴ and spontaneously evolve cholecysto- and hepatolithiasis.²⁰⁵ However, patients with primary biliary cirrhosis have unchanged MDR3 mRNA levels^{206,207} and no MDR3 polymorphisms in PSC patients were observed.²⁰⁸

Dubin-Johnson Syndrome: The Dubin-Johnson syndrome is caused by mutations in MRP2 and the subsequent loss of the canalicular transporter.²⁰⁹⁻²¹¹ This defect results in congenital conjugated hyperbilirubinemia, since MRP2 transports conjugates including bilirubin, divalent bile acids and GSH.^{45,46} Patients with such mutations show an overexpression of basolateral MRP3, which may represent an alternative escape pathway for otherwise accumulating biliary constituents.²¹² Similar, groningen yellow (GY/TR-) wistar rats and Eisai hyperbilirubinemic Sprague Dawley rats (EHBR) lack Mrp2 expression and have chronic conjugated hyperbilirubinemia with reduced bile flow and adaptive overexpression of Mrp3.^{63,213-215}

Genetic defects in basolateral bile acid transporters have so far not been reported, although OATPs appear

to be highly polymorphic.^{3,216} Animal models with Mrp3 and Mrp4 deficiency exist.^{87,217} However, while first results from cholestatic Mrp4 knockout mice are yet not published, bile duct ligation in Mrp3 knockout mice confirmed the only minor role of intestinal Mrp3 for enterohepatic bile acid circulation (see *Osta/β*), the importance of this transport system for regurgitation of conjugated bilirubin from hepatocytes into blood, but surprisingly no protective effect of Mrp3 on liver injury or bile acid levels.²¹⁷

Acquired transporter alterations

Bland cholestasis: Non inflammatory, bland cholestasis can evolve as drug-induced intrahepatic cholestasis (for review²¹⁸) or as intrahepatic cholestasis of pregnancy (ICP) (for review.²¹⁹⁻²²¹) Typical targeted transporters of drug associated cholestasis are BSEP and MRP2. Cyclosporin A induce disorganization of BSEP localization resulting in its intracellular relocation and disruption of the pericanalicular F-actin cytoskeleton²²² and estradiol-17β-D-glucuronide (as model for oral contraceptive-induced cholestasis and ICP) induces endocytotic internalization of Bsep and Mrp2, thus impairing bile acid dependent- and independent bile flow.^{223,224} Also rifamycin SV, rifampicin, glibenclamide and troglitazone cis-inhibit Bsep/BSEP mediated taurocholate transport, thus providing a mechanism for intrahepatic cholestasis caused by these agents.³ In addition, the endothelin antagonist bosentan inhibits BSEP/Bsep but induce Mrp2 mediated biliary bilirubin excretion and bile acid independent bile flow.^{225,226} The development of ICP is multifactorial including potential hepatobiliary transporter affections, sex hormonal maladaptations, dietary and environmental factors. ICP affects up to 2% of pregnancies, has a recurrence rate of 40 to 60%, typically starts with pruritus on palms and soles, and primary bile acids, which are 10 to 100 times higher than in normal pregnancies. The symptoms usually disappears a few days after delivery.²²⁰ While genetic variations in MDR3 (see also PFIC3) may be involved in the pathogenesis of ICP, recently analyzed BSEP sequence variations indicate that this gene might be of minor importance.²²⁷ Also estrogens may contribute to intrahepatic cholestasis of pregnancy and can cause oral-contraceptive-induced cholestasis.^{185,228,229} In the ethinyl estradiol (EE) fed rodent model Mrp2 and Bsep protein is reduced, however, Bsep expression is restored in a long-standing model of EE treatment.²³⁰ These alterations of canalicular transporters is accompanied by a reduction of bile flow of 40%.²³¹ In addition, Na⁺-dependent (Ntcp) and Na⁺-independent (Oatp1) bile acid uptake transporters are also downregulated in this model.²³² EE induced SHP expression might be involved in this downregulation (see above and ref 233). Of note, different baseline expression levels of transporters between genders and in pregnancy may also contribute to a gender specific dis-

ease development or the appearance of a specific disease in pregnancy. As such it was shown that Ntcp was either downregulated in pregnant rats and mice involving downregulation of HNF1 and reduced RAR:RXR binding to the promotor^{234,235} or it was upregulated in postpartum rats involving prolactin mediated Stat5 binding to the Ntcp promotor.²³⁶ Also Oatp2 and Mrp2, Mrp3 and Mrp6 are downregulated in pregnant rats, while in contrast Mrp2 was upregulated in pregnant mice, suggesting species differences under this circumstances.²³⁵⁻²³⁷ Of interest, PXR and its target gene Cyp3a11 were reported to be higher in pregnant mice²³⁸ and CAR to be higher in human females compared to males.²³⁹ Differences between genders were also reported for Oatp1 and Bcrp (higher in females) and Mdr1a and Mrp4 (higher in males) so far.^{235,240}

Inflammatory cholestasis: Clinically, this form of cholestasis is frequently associated with sepsis in the course of intra- and extrahepatic infections, drug- and alcohol induced liver injury, total parenteral nutrition, neoplastic disorders and postoperatively. The common pathophysiological mediator in this entity is endotoxin (lipopolysaccharides (LPS) of the outer bacterial membrane) and/or the release of proinflammatory cytokines (e.g. tumor necrosis factor α (TNFα), interleukin 1β (IL1β)).²⁴¹ In liver biopsies from patients with inflammation-induced cholestasis profound reductions of basolateral NTCP and OATP2 and canalicular BSEP and MRP2 have been observed.²⁴² In the experimental model LPS reduces hepatobiliary transporters at the basolateral and the canalicular membrane, thereby reducing bile acid dependent and independent bile flow. Similar effects were demonstrated for TNFα and IL1β.²⁴³⁻²⁴⁷ LPS leads to a dramatic decrease of Ntcp.^{127,248} The mechanisms involved in this downregulation include reduced binding of HNF1 and RXR:RAR to the rat promotor,^{127,128,249} decline of RXRα, -β, -γ mRNA,²⁵⁰ phosphorylation with reduced activity of RXR via mitogen-activated protein kinase 4 and JNK^{120,121} and reduced HNF4 and 1 activity.^{127,129} SHP does not appear to be involved in LPS mediated Ntcp downregulation.¹²³ Of note, Kupffer cell depletion as well as anti-TNFα and IL1β strategies prevent suppression of Ntcp in endotoxin-treated rats and mice.^{251,252} Endotoxin mediated downregulation of the Na⁺-independent bile acid uptake transporters Oatp1,2,4 involves reduced expression of HNF1,^{129,253,254} reduced expression of PXR and CAR, at least for Oatp2^{83,250,255} and, at least for Oatp4 decreased binding activities of HNF1, RXR:RAR, C/EBP and HNF3.¹³⁵ Bsep expression is only modestly decreased in inflammation-induced cholestasis upon LPS-treatment by 30%,²³⁰ while Mrp2 is profoundly reduced²⁵⁶ by mechanisms including repressed retinoid transactivation via RXR:RAR,¹²⁸ retrieval from the canalicular membrane,²⁵⁷ phosphorylation with subsequent nuclear export of RXR¹²¹ or decreased activity of nuclear receptors FXR, PXR and CAR.²⁵⁸⁻²⁶¹ Mrp3 expression was shown to increase TNFα dependent via upregulation of LRH-1¹⁷³ and

after LPS treatment,^{262,263} however, there are also reports demonstrating downregulation of Mrp3 in response to LPS and TNF α .²⁶⁴

Obstructive cholestasis: Obstructive cholestasis in man may appear as intraluminal obstruction (e.g. gallstones, neoplasias), extraluminal compressions (e.g. neoplasias) biliary atresia, vanishing bile duct syndrome (e.g. primary biliary cirrhosis (PBC) with destruction of small and medium bile ducts) and primary sclerosing cholangitis (PSC) with affection of intra- and extrahepatic large ducts. In patients with extrahepatic biliary atresia NTCP mRNA levels were decreased and subsequently increased if complete biliary drainage by portoenterostomy (Kasai procedure) was achieved²⁶⁵ while BSEP expression was well preserved.²⁶⁶ In patients with biliary obstruction prior to percutaneous transhepatic biliary drainage (PTBD) MRP2, BSEP and MRP3 was reduced.²⁶⁷ Transporter changes in PBC are disease stage dependent: no changes of NTCP, OATP2, BSEP, MDR3 and MRP3 are observed in stage I and II,²⁴² while in the more advanced stages III and IV NTCP and OATP2 are downregulated and MRP3 and MDR1 and 3 are induced.²⁰⁷ This downregulation of import and upregulation of export mechanisms may account for the shift towards renal bile acid elimination in PBC and the increase in bilirubin and serum bile acids in these stages.⁶⁹ BSEP and MRP2 are transiently induced in PBC stage III, potentially aiming to overcome bile acid burden, but return to baseline levels in end stage disease IV.²⁰⁷ In PSC patients however, MRP2 was downregulation, while OATP-A is up-regulated in contrast to downregulation of OATP2.²⁶⁸ One widely used model for obstructive cholestasis is common bile duct ligation (CBDL) in rodents. In these models hepatic uptake systems (i.e. Ntcp, Oatp1) are downregulated,^{269,270} while canalicular export pumps, Bsep and Mrp2 in mice are maintained.²⁷¹ Of note, in rats CBDL results in a marked reduction of Mrp2.²⁵⁶ In addition, basolateral alternative efflux pumps (i.e. Mrp3 and Mrp4) are overexpressed,^{56,271-273} which may shift bile acids and bilirubin from hepatocytes into the systemic circulation. Overexpression of potential renal bile acid export transporters Mrp2 (in rats)⁷¹ and Mrp4 (in mice),⁷² with concomitant reduction of renal re-uptake systems (i.e. Asbt),⁷¹ should enhance alternative elimination via urine. Induced canalicular Asbt may mediate reuptake of bile acids from the obstructed biliary lumen.⁷¹ Mechanism underlying these transporter changes may consist of an inflammatory component since CBDL results in induction of proinflammatory cytokines²⁷⁴⁻²⁷⁶ and the accumulation of endogenous bile acids and biliary compounds (e.g. bilirubin) which activate several nuclear receptors (e.g. FXR, PXR, VDR, CAR). Activation of FXR by accumulating bile acids induces SHP and may downregulate Ntcp.^{123,252} Alternatively, it has been shown that bile acids are also capable of inducing SHP via an FXR-inde-

pendent mechanism involving JNK.¹²⁶ Bile acids have also been shown to suppress HNF4 transcription,^{90,91} which might reduce HNF1 and subsequently Ntcp. Systemic proinflammatory cytokines have no effect on Ntcp expression during obstructive cholestasis since TNF α and IL-1 β antagonists have no effects on Ntcp expression.²⁵² However, Mrp2 downregulation in rats was attributed to cytokine dependent inhibition of RAR:RXR binding to the Mrp2 promotor.²⁷⁷ TNF α induced LRH-1 as well as reduced RAR:RXR might be involved in the upregulation of Mrp3.^{170,173} In addition activation of PXR and CAR by bile acids and/or bilirubin might contribute to the observed changes.^{149,163} Similar mechanisms may play a role in the induction of Mrp4 via CAR.^{86,87} The Mdr2 knockout mouse represents an excellent model for studying sclerosing cholangitis^{204,278} and long term effects of cholestasis on transporter expression. Mdr2 knockout mice show persistent downregulation of Ntcp, but this is obviously not sufficient to prevent intrahepatic bile acid accumulation. Moreover, Mdr2 knockout mice do not show adaptive upregulation of alternative basolateral Mrp3 and Mrp4 expression when the cholestatic phenotype is fully developed, although intrahepatic bile acid levels remain increased.²⁷⁹ A model for non surgical intrahepatic reversible cholestasis is administration of α -naphthylisothiocyanate (ANIT).^{280,281} A recent study using rats found a common cholestatic transporter pattern with downregulation of Ntcp and Oatp1, while Bsep remained unchanged and Mdr2 as well as Mrp3 were upregulated.²⁸²

Therapeutic options 2005 and beyond

Counteracting cholestatic disorders should be a multi-level approach to target impaired physiological functions and pathophysiological mechanisms of bile acid/bilirubin transport and metabolism and to create/support adaptive rescue pathways for potential toxic accumulating biliary compounds. Unraveling the mechanism and "players" lying behind physiological and pathophysiological cholestatic conditions allows a better understanding of empiric treatment strategies (i.e. UDCA, rifampicin (Rifa), Phenobarbital (PB)) and the development of novel, more effective treatment options customized to the special requirements of the proper cholestatic disorder (*Table II*).

Ursodeoxycholic acid: To date UDCA is the only approved drug for treatment of PBC and PSC, since UDCA was shown to improve biochemical and clinical parameters in a variety of cholestatic syndromes and positively effects survival free of liver transplantation in PBC.^{283,284} The mechanisms of action of UDCA are multi-level comprising transcriptional transporter induction and targeting of bile acid metabolizing enzymes (see below), posttranscriptional transporter trafficking followed by increased insertion of canalicular transport systems (Bsep) into the canalicular membrane of rats and stimulating of transporter function by inducing phosphorylation of ABC

Table II. Nuclear receptors agonists and their expected effects in cholestatic liver disease.

Nuclear receptor	Ligands	Target genes	Expected/proposed effect
FXR (NR1H4)	CDCA, CA, GW4064, 6-ECDCA, partially UDCA	SHP, BSEP, I-BABP, MRP2, OATP8; indirectly NTCP, OATP2, CYP7A1, CYP8B1	Reducing hepatotoxic bile acid load in cholestatic hepatocytes by enhancing bile acid dependent and independent bile flow, shutting down mayor bile acid uptake systems, and reducing endogenous bile acid synthesis
PXR (NR1I2)	Rifampicin (human), PCN (rodent), dexamethasone, LCA, potentially UDCA, phenobarbital (human), statins, St. John's wort, xenobiotics	MRP2, MRP3, Oatp2, MDR1, CYP2B, CYP3A, CYP7A1, SULT2A1, UGT1A1	Reducing hepatotoxic bile acid load in cholestatic hepatocytes by enhancing basolateral alternative bile acid efflux, detoxifying bile acids via hydroxilation and sulfatation, and reducing endogenous bile acid synthesis as well as stimulation of bilirubin glucuronidation and excretion
CAR (NR1I3)	Bilirubin, phenobarbital, TCPOBOP (rodent), CITCO (human), dimethoxycoumarin, xenobiotics	MRP2, MRP3, MRP4, CYP2B, CYP3A, SULT2A1, UGT1A1	Reducing hepatotoxic bile acid load in cholestatic hepatocytes by enhancing alternative basolateral bile acid efflux, detoxifying bile acid via hydroxilation and sulfatation as well as stimulation of bilirubin glucuronidation and excretion
PPAR α (NR1C1)	Fatty acids, fibrates, statins, eicasonoids, leukotriens, NSAIDs, WY-14643	Mdr2, ASBT, CYP7A1, CYP8B1, UGT2B4, SULT2A1	Protection of bile duct epithelium via increased phospholipid secretion, reducing endogenous bile acid synthesis and detoxifying bile acids via glucuronidation
GR (NR3C1)	Glucocorticoids, potentially UDCA	CAR, ASBT, NTCP, potentially AE2, BSEP, MRP2	Beneficial effects in cholestatic jaundice ("steroid whitewash") may be mediated by direct transporter effects or antiinflammatory effects

NOTE. CA, cholic acid; CDCA, chenodeoxycholic acid; CITCO, 6-(4-chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-ichlorobenzyl)oxime; 6-ECDCA, 6-ethyl chenodeoxycholic acid; LCA, lithocholic acid; NSAIDs, non steroidal antiinflammatory drugs; PCN, pregnenolone 16 α -carbonitrile; TCPOBOP, 1,4-bis-[2-(3,5-dichlorpyridyloxy)]benzene; UDCA, ursodeoxycholic acid.

²⁸⁵ while additionally converting a hydrophobic bile acid pool into a more hydrophilic one (for review).^{283,284,286} Most of the knowledge however, is obtained from experiments in animals. Albeit UDCA changes transcriptionally gene expression,^{67,287} no definite nuclear receptor has been elucidated. Of note, FXR and PXR may mediate some of the UDCA actions,^{98,138} but most of the transcriptional transporter effects are FXR independent.^{67,271} UDCA stimulates bile acid dependent and independent bile flow in naïve mice via induction of canalicular Bsep and Mrp2.^{67,287} This "orthograde" biliary excretion is extended by induction of additional adaptive basolateral efflux pumps Mrp3 and Mrp4, which are suggested to efflux bile acids "retrogradely" back into the systemic circulation followed by their renal elimination.^{67,288} Stimulation of renal (Mrp2, Mrp4) and intestinal (Mrp2, Mrp3) bile acid efflux pumps might support the elimination of potentially toxic biliary constituents in other bile acid targeted tissues, thus contributing to an overall enhanced bile acid elimination.⁶⁷ While parts of these adaptive transporter alterations are already intrinsically induced in cholestatic animal models^{72,271} (Tauro)UDCA failed to additionally stimulate these transporters to significant extents in cholestatic mice²⁸² (and Wagner and Trauner, unpublished observations) demanding the use of more potent therapeutical agents. In healthy humans UDCA induces BSEP and MRP4 expression on posttranscriptional levles.²⁸⁹ In PBC UDCA enrichment correlated positively with OATP1B1 and MRP2,²⁰⁷ thus potentially contributing to reduced bilirubin and bile acid levels ob-

served in these patients.^{283,286} On metabolic levels UDCA has been shown to stimulate bile acid hydroxylating CYP3A4/Cyp3a11 in human and rodents^{138,290} and to downregulate human CYP7A1 the key enzyme of bile acid synthesis *in vitro*.^{98,291} Thus, UDCA appears to act on transporter level by restoring defective transporters (e.g. Bsep, Mrp2) and generating alternative overflow-systems (Mrp3, Mrp4) for accumulating biliary compounds and on metabolic level by induction of detoxification pathways (Cyp3a11).

FXR agonists: Pharmaceutical compounds targeting FXR are promising therapeutical approaches since studies in humans and rodents revealed a predominant role of disrupted FXR in diverse aspects of cholestasis related disorders. As such FXR was reported to be downregulated upon CA challenge,¹¹⁶ endogenous bile acid load in CBDL¹¹⁵ and in response to proinflammatory cytokines (TNF α and IL-1) and endotoxin.²⁵⁸ PFIC1 is associated with markedly reduced FXR levels¹¹⁷ and PFIC2 patients lack the typical primary FXR target BSEP.⁴³ Moreover, FXR^{-/-} mice exhibit severe liver injury and mortality after CA feeding,^{67,112} which could be explained by the lack of downregulation of the bile acid uptake systems Ntcp and export pump Bsep as well as the lack in shutting down endogenous bile acid formation via Cyp7a1 and Cyp8b1.¹¹² However, CBDL in FXR^{-/-} did not result in aggravation of liver injury.²⁷¹ This effect may be attributed in part to the induction of FXR independent alternative hepatic (Mrp3, Mrp4) and renal (Mrp2, Mrp4) export pumps and induction of bile acid detoxification which are

higher in FXR^{-/-} (Cyp3a11 and Sult2a1).^{72,108,138,271,292} FXR agonists, thus should overcome cholestatic disorders with reduced bile flow via stimulation of Bsep (increasing bile acid-dependent bile flow) and Mrp2 (increasing bile acid-independent bile flow), reducing bile acid uptake by downregulating Ntcp and Oatp1 and bile acid formation via reduction of Cyp7a1 and Cyp8b1.^{67,97,102,112,270,291,293} Induction of MDR3 via FXR might protect the bile duct by increasing the biliary phospholipid content.¹¹⁰ However, in the presence of biliary obstruction a choleretic dose of UDCA (a partial FXR agonist, which induces Bsep)⁹⁸ in CBDL mice results in increased biliary pressure with rupture of cholangoles resulting in aggravation of bile infarcts and an increased mortality.²⁰⁴ Unfortunately, many clinically relevant chronic cholestatic liver diseases such PSC have a significant obstructive component, thus lowering the therapeutical use of FXR agonists in advanced disease stages. First experiments with synthetic FXR agonists in cholestatic rodent models have been promising and resulted in reduced biochemical and histomorphological markers of liver injury in some models.^{282,294,295} Noteworthy are also the antifibrotic effects of the strong half-synthetic FXR agonist 6-ECDCA *in vitro* and *in vivo*, showing that FXR may be a negative regulator of hepatic stellate cells via SHP and the AP-1 transcription factor complex.²⁹⁶ However, the therapeutical use of FXR agonists could be limited because of interfering regulatory functions of FXR in lipid homeostasis, particularly in HDL and triglyceride metabolism.²⁹³

PXR and CAR agonists: In contrast to FXR which is downregulated under cholestatic conditions^{115,116} (and Wagner, Trauner unpublished observations) PXR and CAR remain almost unchanged after bile acid treatment¹¹⁶ thus providing ideal therapeutical targets in cholestasis. Long before knowing their exact mode of action PXR (rifampicin) and CAR (phenobarbital) ligands have been used for treatment of cholestatic disorders and symptoms (jaundice, pruritus). However, in most cases effects on cholestatic parameters in PBC were only minor.²⁹⁷⁻³⁰⁴ The potent rodent PXR ligand PCN was reported to protect against LCA induced liver injury on various levels, effects, which may be mediated by induction of Cyp3a11,⁸² induction of Oatp2 and downregulation of Cyp7a1,⁸³ moreover by induction of bile acid sulfating sulfotransferase (Sult2a1) together with its sulfonyl donor 3'-phosphoadenosine 5'-phosphosulfate synthetase 2 (PAPSS2).¹⁵³ In addition, PXR activation might contribute to reduced injury by its effects on bilirubin glucuronidating Ugt1a1 and exporting Mrp2.^{102,154} Similar to FXR stimulation it was suggested that PCN also has antifibrotic effects by reducing α -smooth muscle antigen and collagen as fibrotic markers.³⁰⁵

In addition to phenobarbital as prototypic CAR activator certain compounds of traditional Chinese herbs, which have long been used for treatment of neonatal jaundice in humans were found to be potent CAR activators.¹⁶⁵ Stimulation of CAR in mice drastically reduced serum levels

of infused bilirubin via induction of bilirubin uptake (Slc21a6), glucuronidation of bilirubin (Ugt1a1) and canalicular excretion via Mrp2.⁸⁶ In mouse models of cholestasis CAR agonists were shown to defend LCA induced toxicity primarily by induction of Sult2a and PAPSS2 gene expression with subsequent LCA sulfation and of minor importance again via induction of Cyp3a11 with subsequent bile acid hydroxylation.¹⁶⁴ Also the stimulatory effects of CAR on Mrp3 and sulfated bile acid transporting Mrp4 together with coordinated induction of Sult2a1 might contribute to the beneficial effects of CAR ligands.^{87,163} This fundamental role of CAR in bile acid detoxification is further strengthened by a study exploring CA toxicity in FXR^{-/-} and PXR^{-/-}, which revealed that the use of CAR agonists can mitigate CA induced toxicity even when both classical bile acid receptors are knocked-out.¹¹⁶ In summary, the use of specific agonists for PXR or CAR should counteract hepatotoxicity of accumulated bile acids and bilirubin by a orchestra of hydroxylation (via Cyp3a11 and potentially Cyp2b10), sulfation (via Sult2a1 and PAPSS2) and glucuronidation (via Ugt1a1) reactions as well as coordinated induction of transporters (Mrp3, Mrp4) for these substrates. However these drugs may also toxify certain previously un toxic compounds to their toxic forms,^{163,306} enhance metabolism of other administered drugs or may interact endogenous metabolic pathways.³⁰⁷ Moreover, it should be noted that the liver tumor promoting potential of PB is directly linked to the presence of CAR³⁰⁸ and that PXR might play a role in the biology of endometrial cancer.³⁰⁹

PPAR α agonists: The rationale for the therapeutic use of PPAR α agonists in cholestasis is given by the observations that fibrates or statins, both being ligands for PPAR α showed beneficial effects on biochemical parameters of cholestasis and/or transaminases.³¹⁰⁻³¹³ The beneficial effects could at least in part be explained by the potential to stimulate canalicular phospholipid flipping via Mdr2 and thus protecting the bile duct epithelium from detergent effects of bile acids by enhanced biliary phospholipids secretion and formation of mixed micelles.^{175,314-317} In addition, repression of CYP7A1 (so far only shown for fibrates),³¹⁸ or pleiotropic anti-inflammatory effects may contribute.³¹⁹

Corticosteroids: The beneficial effects of corticosteroids (e.g. prednisolone or budesonide), which have been used in acute cholestasis (e.g., "steroid white-wash" in drug- and inflammation-induced cholestasis) as well as chronic cholestatic disorders such as PBC³²⁰⁻³²² could be attributed to their anti-inflammatory and immune-modulatory effects and, at least in part, to effects mediated via transporter and enzyme alterations. As such, corticosteroids were reported to induce Mrp2 and Bsep and counteract its downregulation after endotoxin treatment *in vitro*.^{257,323} These effects could possibly mediated by targeting the glucocorticoid receptor, although this has so far only been shown for intestinal ASBT³²⁴ and NTCP.¹⁸¹

In summary, targeting nuclear receptors with specific potent agonists is a promising new therapeutic option for modulating distinct bile acids and/or bilirubin pathways. The development of gene-selective agonists will potentially allow the modulation of single transporter and detoxification systems depending on the given requirements in future.

Summary and outlook

Hereditary and acquired alterations in hepatobiliary transporter systems may result in the development or maintenance of cholestatic disorders. On the other hand adaptive induction of overflow and detoxification systems by the affected hepatocyte results in the primary intrinsic attempt to counteract liver injury. However, in chronic cholestasis these adaptations are not sufficient. The increasing information on the molecular mechanisms of bile acid transport and metabolism under physiological and pathophysiological conditions may guide the development of novel, more effective drugs. Nuclear receptors and their target genes are promising treatment options for cholestatic liver diseases.

References

- Hagenbuch B, Stieger B, Foguet M, Lubbert H, Meier PJ. Functional expression cloning and characterization of the hepatocyte Na⁺/bile acid cotransport system. *Proc Natl Acad Sci U S A* 1991; 88(23): 10629-33.
- Trauner M, Boyer JL. Bile salt transporters: molecular characterization, function, and regulation. *Physiol Rev* 2003; 83(2): 633-71.
- Kullak-Ublick GA, Stieger B, Meier PJ. Enterohepatic bile salt transporters in normal physiology and liver disease. *Gastroenterology* 2004; 126(1): 322-42.
- Meier PJ, Stieger B. Bile salt transporters. *Annu Rev Physiol* 2002; 64: 635-61.
- Jansen PL, Sturm E. Genetic cholestasis, causes and consequences for hepatobiliary transport. *Liver Int* 2003; 23(5): 315-22.
- Trauner M, Meier PJ, Boyer JL. Molecular pathogenesis of cholestasis. *N Engl J Med* 1998; 339(17): 1217-27.
- Anwer MS. Cellular regulation of hepatic bile acid transport in health and cholestasis. *Hepatology* 2004; 39(3): 581-90.
- Eloranta JJ, Kullak-Ublick GA. Coordinate transcriptional regulation of bile acid homeostasis and drug metabolism. *Arch Biochem Biophys* 2005; 433(2): 397-412.
- Karpen SJ. Nuclear receptor regulation of hepatic function. *J Hepatol* 2002; 36(6): 832-50.
- Wolkoff AW, Cohen DE. Bile acid regulation of hepatic physiology: I. Hepatocyte transport of bile acids. *Am J Physiol Gastrointest Liver Physiol* 2003; 284(2): G175-G179.
- Chiang JY. Bile acid regulation of hepatic physiology: III. Bile acids and nuclear receptors. *Am J Physiol Gastrointest Liver Physiol* 2003; 284(3): G349-G356.
- Hofmann AF. The continuing importance of bile acids in liver and intestinal disease. *Arch Intern Med* 1999; 159(22): 2647-58.
- Kullak-Ublick GA, Stieger B, Hagenbuch B, Meier PJ. Hepatic transport of bile salts. *Semin Liver Dis* 2000; 20(3): 273-92.
- Hofmann AF. Bile Acids: The Good, the Bad, and the Ugly. *News Physiol Sci* 1999; 14: 24-9.
- Hofmann AF. Cholestatic liver disease: pathophysiology and therapeutic options. *Liver* 2002; 22 Suppl 2: 14-9.
- Chiang JY. Bile acid regulation of gene expression: roles of nuclear hormone receptors. *Endocr Rev* 2002; 23(4): 443-63.
- De FE, Mitro N, Godio C, Gilardi F, Caruso D, Crestani M. Bile acid signaling to the nucleus: finding new connections in the transcriptional regulation of metabolic pathways. *Biochimie* 2004; 86(11): 771-8.
- Chiang JY. Regulation of bile acid synthesis: pathways, nuclear receptors, and mechanisms. *J Hepatol* 2004; 40(3): 539-51.
- Russell DW. The enzymes, regulation, and genetics of bile acid synthesis. *Annu Rev Biochem* 2003; 72: 137-74.
- Duane WC, Javitt NB. 27-hydroxycholesterol: production rates in normal human subjects. *J Lipid Res* 1999; 40(7): 1194-9.
- Axelsson M, Sjoval J. Potential bile acid precursors in plasma—possible indicators of biosynthetic pathways to cholic and chenodeoxycholic acids in man. *J Steroid Biochem* 1990; 36(6): 631-40.
- Radominska-Pyrek A, Zimniak P, Irshaid YM, Lester R, Tephly TR, St PJ. Glucuronidation of 6 alpha-hydroxy bile acids by human liver microsomes. *J Clin Invest* 1987; 80(1): 234-41.
- Takikawa H, Otsuka H, Beppu T, Seyama Y, Yamakawa T. Serum concentrations of bile acid glucuronides in hepatobiliary diseases. *Digestion* 1983; 27(4): 189-95.
- Marschall HU, Matern H, Egestad B, Matern S, Sjoval S. 6 alpha-glucuronidation of hyodeoxycholic acid by human liver, kidney and small bowel microsomes. *Biochim Biophys Acta* 1987; 921(2): 392-7.
- van Berge Henegouwen GP, Brandt KH, Eyssen H, Parmentier G. Sulphated and unsulphated bile acids in serum, bile, and urine of patients with cholestasis. *Gut* 1976; 17(11): 861-9.
- Radominska A, Comer KA, Zimniak P, Falany J, Iscan M, Falany CN. Human liver steroid sulphotransferase sulphates bile acids. *Biochem J* 1990; 272(3): 597-604.
- Palmer RH. The formation of bile acid sulfates: a new pathway of bile acid metabolism in humans. *Proc Natl Acad Sci USA* 1967; 58(3): 1047-50.
- Meier PJ. Molecular mechanisms of hepatic bile salt transport from sinusoidal blood into bile. *Am J Physiol* 1995; 269(6 Pt 1): G801-G812.
- Arias IM. The biology of hepatic endothelial cell fenestrae. *Prog Liver Dis* 1990; 9: 11-26.
- Berk PD, Potter BJ, Stremmel W. Role of plasma membrane ligand-binding proteins in the hepatocellular uptake of albumin-bound organic anions. *Hepatology* 1987; 7(1): 165-76.
- Reichen J. The Role of the Sinusoidal Endothelium in Liver Function. *News Physiol Sci* 1999; 14: 117-21.
- Layden TJ, Boyer JL. Influence of bile acids on bile canalicular membrane morphology and the lobular gradient in canalicular size. *Lab Invest* 1978; 39(2): 110-9.
- Meier PJ, Eckhardt U, Schroeder A, Hagenbuch B, Stieger B. Substrate specificity of sinusoidal bile acid and organic anion uptake systems in rat and human liver. *Hepatology* 1997; 26(6): 1667-77.
- Hagenbuch B, Meier PJ. The superfamily of organic anion transporting polypeptides. *Biochim Biophys Acta* 2003; 1609(1): 1-18.
- St Pierre MV, Kullak-Ublick GA, Hagenbuch B, Meier PJ. Transport of bile acids in hepatic and non-hepatic tissues. *J Exp Biol* 2001; 204(Pt 10): 1673-86.
- Hirohashi T, Suzuki H, Takikawa H, Sugiyama Y. ATP-dependent transport of bile salts by rat multidrug resistance-associated protein 3 (Mrp3). *J Biol Chem* 2000; 275(4): 2905-10.
- Zelcer N, Reid G, Wielinga P, Kuil A, van dH, I, Schuetz JD, Borst P. Steroid and bile acid conjugates are substrates of human multidrug-resistance protein (MRP) 4 (ATP-binding cassette C4). *Biochem J* 2003; 371(Pt 2): 361-7.
- Rius M, Nies AT, Hummel-Eisenbeiss J, Jedlitschky G, Keppler D. Cotransport of reduced glutathione with bile salts by MRP4 (ABCC4) localized to the basolateral hepatocyte membrane. *Hepatology* 2003; 38(2): 374-84.
- Li L, Meier PJ, Ballatori N. Oatp2 mediates bidirectional organic solute transport: a role for intracellular glutathione. *Mol Pharmacol* 2000; 58(2): 335-40.
- Seward DJ, Koh AS, Boyer JL, Ballatori N. Functional complementation between a novel mammalian polygenic transport complex and an evolutionarily ancient organic solute transporter, OSTalpha-OSTbeta. *J Biol Chem* 2003; 278(30): 27473-82.

41. Dawson PA, Hubbert M, Haywood J, Craddock AL, Zerangue N, Christian WV, Ballatori N. The Heteromeric Organic Solute Transporter {alpha}-{beta}, Ost{alpha}-Ost{beta}, Is an Ileal Basolateral Bile Acid Transporter. *J Biol Chem* 2005; 280(8): 6960-8.
42. Agellon LB, Torchia EC. Intracellular transport of bile acids. *Biochim Biophys Acta* 2000; 1486(1): 198-209.
43. Jansen PL, Strautnieks SS, Jacquemin E, Hadchouel M, Sokal EM, Hooiveld GJ, Koning JH, et al. Hepatocanicular bile salt export pump deficiency in patients with progressive familial intrahepatic cholestasis. *Gastroenterology* 1999; 117(6): 1370-9.
44. Wang R, Salem M, Yousef IM, Tuchweber B, Lam P, Childs SJ, Helgason CD, et al. Targeted inactivation of sister of P-glycoprotein gene (spgp) in mice results in nonprogressive but persistent intrahepatic cholestasis. *Proc Natl Acad Sci USA* 2001; 98(4): 2011-6.
45. Akita H, Suzuki H, Ito K, Kinoshita S, Sato N, Takikawa H, Sugiyama Y. Characterization of bile acid transport mediated by multidrug resistance associated protein 2 and bile salt export pump. *Biochim Biophys Acta* 2001; 1511(1): 7-16.
46. Keppler D, König J. Hepatic secretion of conjugated drugs and endogenous substances. *Semin Liver Dis* 2000; 20(3): 265-72.
47. Suzuki M, Suzuki H, Sugimoto Y, Sugiyama Y. ABCG2 transports sulfated conjugates of steroids and xenobiotics. *J Biol Chem* 2003; 278(25): 22644-9.
48. Smit JJ, Schinkel AH, Oude Elferink RP, Groen AK, Wagenaar E, van DL, Mol CA, et al. Homozygous disruption of the murine mdr2 P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease. *Cell* 1993; 75(3): 451-62.
49. Yu L, Hammer RE, Li-Hawkins J, von BK, Lutjohann D, Cohen JC, Hobbs HH. Disruption of Abcg5 and Abcg8 in mice reveals their crucial role in biliary cholesterol secretion. *Proc Natl Acad Sci USA* 2002; 99(25): 16237-42.
50. Meier PJ, Knickelbein R, Moseley RH, Dobbins JW, Boyer JL. Evidence for carrier-mediated chloride/bicarbonate exchange in canalicular rat liver plasma membrane vesicles. *J Clin Invest* 1985; 75(4): 1256-63.
51. Kanno N, Lesage G, Glaser S, Alvaro D, Alpini G. Functional heterogeneity of the intrahepatic biliary epithelium. *Hepatology* 2000; 31(3): 555-61.
52. Hofmann AF. The cholehepatic circulation of unconjugated bile acids: an update. In: Paumgartner G, Stiehl A, Gerok W, editors. *Bile acids and the hepatobiliary system*. Boston: Kluwer; 1993. p.143-60.
53. Lazaridis KN, Pham L, Tietz P, Marinelli RA, deGroen PC, Levine S, Dawson PA, et al. Rat cholangiocytes absorb bile acids at their apical domain via the ileal sodium-dependent bile acid transporter. *J Clin Invest* 1997; 100(11): 2714-21.
54. Benedetti A, Di SA, Marucci L, Svegliati-Baroni G, Schteingart CD, Ton-Nu HT, Hofmann AF. Carrier-mediated transport of conjugated bile acids across the basolateral membrane of biliary epithelial cells. *Am J Physiol* 1997; 272(6 Pt 1): G1416-G1424.
55. Chignard N, Mergey M, Veissiere D, Parc R, Capeau J, Poupon R, Paul A, et al. Bile acid transport and regulating functions in the human biliary epithelium. *Hepatology* 2001; 33(3): 496-503.
56. Soroka CJ, Lee JM, Azzaroli F, Boyer JL. Cellular localization and up-regulation of multidrug resistance-associated protein 3 in hepatocytes and cholangiocytes during obstructive cholestasis in rat liver. *Hepatology* 2001; 33(4): 783-91.
57. Kool M, van der LM, de HM, Scheffer GL, de Vree JM, Smith AJ, Jansen G, et al. MRP3, an organic anion transporter able to transport anti-cancer drugs. *Proc Natl Acad Sci USA* 1999; 96(12): 6914-9.
58. Lazaridis KN, Tietz P, Wu T, Kip S, Dawson PA, LaRusso NF. Alternative splicing of the rat sodium/bile acid transporter changes its cellular localization and transport properties. *Proc Natl Acad Sci USA* 2000; 97(20): 11092-7.
59. Craddock AL, Love MW, Daniel RW, Kirby LC, Walters HC, Wong MH, Dawson PA. Expression and transport properties of the human ileal and renal sodium-dependent bile acid transporter. *Am J Physiol* 1998; 274(1 Pt 1): G157-G169.
60. Walters HC, Craddock AL, Fusegawa H, Willingham MC, Dawson PA. Expression, transport properties, and chromosomal location of organic anion transporter subtype 3. *Am J Physiol Gastrointest Liver Physiol* 2000; 279(6): G1188-G1200.
61. Amelsberg A, Jochims C, Richter CP, Nitsche R, Folsch UR. Evidence for an anion exchange mechanism for uptake of conjugated bile acid from the rat jejunum. *Am J Physiol* 1999; 276(3 Pt 1): G737-G742.
62. Gong YZ, Everett ET, Schwartz DA, Norris JS, Wilson FA. Molecular cloning, tissue distribution, and expression of a 14-kDa bile acid-binding protein from rat ileal cytosol. *Proc Natl Acad Sci USA* 1994; 91(11): 4741-5.
63. Hirohashi T, Suzuki H, Ito K, Ogawa K, Kume K, Shimizu T, Sugiyama Y. Hepatic expression of multidrug resistance-associated protein-like proteins maintained in eisai hyperbilirubinemic rats. *Mol Pharmacol* 1998; 53(6): 1068-75.
64. Zeng H, Liu G, Rea PA, Kruh GD. Transport of amphipathic anions by human multidrug resistance protein 3. *Cancer Res* 2000; 60(17): 4779-84.
65. Cherrington NJ, Hartley DP, Li N, Johnson DR, Klaassen CD. Organ distribution of multidrug resistance proteins 1, 2, and 3 (Mrp1, 2, and 3) mRNA and hepatic induction of Mrp3 by constitutive androstane receptor activators in rats. *J Pharmacol Exp Ther* 2002; 300(1): 97-104.
66. Rost D, Mahner S, Sugiyama Y, Stremmel W. Expression and localization of the multidrug resistance-associated protein 3 in rat small and large intestine. *Am J Physiol Gastrointest Liver Physiol* 2002; 282(4): G720-G726.
67. Zollner G, Fickert P, Fuchsichler A, Silbert D, Wagner M, Arbeiter S, Gonzalez FJ, et al. Role of nuclear bile acid receptor, FXR, in adaptive ABC transporter regulation by cholic and ursodeoxycholic acid in mouse liver, kidney and intestine. *J Hepatol* 2003; 39(4): 480-8.
68. Wilson FA, Burckhardt G, Murer H, Rumrich G, Ullrich KJ. Sodium-coupled taurocholate transport in the proximal convoluted of the rat kidney *in vivo* and *in vitro*. *J Clin Invest* 1981; 67(4): 1141-50.
69. Raedsch R, Lauterburg BH, Hofmann AF. Altered bile acid metabolism in primary biliary cirrhosis. *Dig Dis Sci* 1981; 26(5): 394-401.
70. Bergwerk AJ, Shi X, Ford AC, Kanai N, Jacquemin E, Burk RD, Bai S, et al. Immunologic distribution of an organic anion transport protein in rat liver and kidney. *Am J Physiol* 1996; 271(2 Pt 1): G231-G238.
71. Lee J, Azzaroli F, Wang L, Soroka CJ, Gigliozi A, Setchell KD, Kramer W, et al. Adaptive regulation of bile salt transporters in kidney and liver in obstructive cholestasis in the rat. *Gastroenterology* 2001; 121(6): 1473-84.
72. Wagner M, Fickert P, Marschall HU, Zollner G, Silbert D, Fuchsichler A, Gumhold J, et al. Alternative Excretory Routes for Accumulating Bile Acids via Adaptive Renal ABC Transporter Expression in Common Bile Duct-ligated (CBDL) Mice. *Gastroenterology* 2004; 126[4, Suppl. 2], A-129.
73. Kullak-Ublick GA, Hagenbuch B, Stieger B, Schteingart CD, Hofmann AF, Wolkoff AW, Meier PJ. Molecular and functional characterization of an organic anion transporting polypeptide cloned from human liver. *Gastroenterology* 1995; 109(4): 1274-82.
74. Schaub TP, Kartenbeck J, König J, Spring H, Dorsam J, Staehler G, Storkel S, et al. Expression of the MRP2 gene-encoded conjugate export pump in human kidney proximal tubules and in renal cell carcinoma. *J Am Soc Nephrol* 1999; 10(6): 1159-69.
75. van Aubel RA, Smeets PH, Peters JG, Bindels RJ, Russel FG. The MRP4/ABCC4 gene encodes a novel apical organic anion transporter in human kidney proximal tubules: putative efflux pump for urinary cAMP and cGMP. *J Am Soc Nephrol* 2002; 13(3): 595-603.
76. Soroka C, Chi SY, Boyer J L. Effects of cholestasis on the regulation of membrane transporter expression in intestine and kidney. *Hepatology* 2002; 36[4, Suppl 2], 1195.
77. Handschin C, Meyer UA. Induction of drug metabolism: the role of nuclear receptors. *Pharmacol Rev* 2003; 55(4): 649-73.
78. Xu L, Glass CK, Rosenfeld MG. Coactivator and corepressor complexes in nuclear receptor function. *Curr Opin Genet Dev* 1999; 9(2): 140-7.
79. Parks DJ, Blanchard SG, Bledsoe RK, Chandra G, Consler TG, Kliewer SA, Stimmel JB, et al. Bile acids: natural ligands for an orphan nuclear receptor. *Science* 1999; 284(5418): 1365-8.

80. Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, Hull MV, et al. Identification of a nuclear receptor for bile acids. *Science* 1999; 284(5418): 1362-5.
81. Wang H, Chen J, Hollister K, Sowers LC, Forman BM. Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. *Mol Cell* 1999; 3(5): 543-53.
82. Xie W, Radominska-Pandya A, Shi Y, Simon CM, Nelson MC, Ong ES, Waxman DJ, et al. An essential role for nuclear receptors SXR/PXR in detoxification of cholestatic bile acids. *Proc Natl Acad Sci USA* 2001; 98(6): 3375-80.
83. Staudinger JL, Goodwin B, Jones SA, Hawkins-Brown D, MacKenzie KI, LaTour A, Liu Y, et al. The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. *Proc Natl Acad Sci USA* 2001; 98(6): 3369-74.
84. Makishima M, Lu TT, Xie W, Whitfield GK, Domoto H, Evans RM, Haussler MR, et al. Vitamin D receptor as an intestinal bile acid sensor. *Science* 2002; 296(5571): 1313-6.
85. Song C, Hiipakka RA, Liao S. Selective activation of liver X receptor alpha by 6alpha-hydroxy bile acids and analogs. *Steroids* 2000; 65(8): 423-7.
86. Huang W, Zhang J, Chua SS, Qatanani M, Han Y, Granata R, Moore DD. Induction of bilirubin clearance by the constitutive androstane receptor (CAR). *Proc Natl Acad Sci USA* 2003; 100(7): 4156-61.
87. Assem M, Schuetz EG, Leggas M, Sun D, Yasuda K, Reid G, Zelcer N, et al. Interactions between hepatic Mrp4 and Sult2a as revealed by the constitutive androstane receptor and Mrp4 knockout mice. *J Biol Chem* 2004; 279(21): 22250-7.
88. Mangelsdorf DJ, Evans RM. The RXR heterodimers and orphan receptors. *Cell* 1995; 83(6): 841-50.
89. Tzamelis I, Chua SS, Cheskis B, Moore DD. Complex effects of rexinoids on ligand dependent activation or inhibition of the xenobiotic receptor, CAR. *Nucl Recept* 2003; 1(1): 2.
90. Davis RA, Miyake JH, Hui TY, Spann NJ. Regulation of cholesterol-7alpha-hydroxylase: BAREly missing a SHP. *J Lipid Res* 2002; 43(4): 533-43.
91. Jung D, Kullak-Ublick GA. Hepatocyte nuclear factor 1 alpha: a key mediator of the effect of bile acids on gene expression. *Hepatology* 2003; 37(3): 622-31.
92. Jung D, Hagenbuch B, Fried M, Meier PJ, Kullak-Ublick GA. Role of liver-enriched transcription factors and nuclear receptors in regulating the human, mouse, and rat NTCP gene. *Am J Physiol Gastrointest Liver Physiol* 2004; 286(5): G752-G761.
93. Lu TT, Repa JJ, Mangelsdorf DJ. Orphan nuclear receptors as eLXR and FiXR of sterol metabolism. *J Biol Chem* 2001; 276(41): 37735-8.
94. Otte K, Kranz H, Kober I, Thompson P, Hoefer M, Haubold B, Rimmel B, et al. Identification of farnesoid X receptor beta as a novel mammalian nuclear receptor sensing lanosterol. *Mol Cell Biol* 2003; 23(3): 864-72.
95. Huber RM, Murphy K, Miao B, Link JR, Cunningham MR, Rupa MJ, Gunyuzlu PL, et al. Generation of multiple farnesoid-X-receptor isoforms through the use of alternative promoters. *Gene* 2002; 290(1-2): 35-43.
96. Bishop-Bailey D, Walsh DT, Warner TD. Expression and activation of the farnesoid X receptor in the vasculature. *Proc Natl Acad Sci USA* 2004; 101(10): 3668-73.
97. Yu J, Lo JL, Huang L, Zhao A, Metzger E, Adams A, Meinke PT, et al. Lithocholic acid decreases expression of bile salt export pump through farnesoid X receptor antagonist activity. *J Biol Chem* 2002; 277(35): 31441-7.
98. Lew JL, Zhao A, Yu J, Huang L, de PN, Pelaez F, Wright SD, et al. The farnesoid X receptor controls gene expression in a ligand- and promoter-selective fashion. *J Biol Chem* 2004; 279(10): 8856-61.
99. Karpen SJ. Bile acids go nuclear! *Hepatology* 1999; 30(4): 1107-9.
100. Laffitte BA, Kast HR, Nguyen CM, Zavacki AM, Moore DD, Edwards PA. Identification of the DNA binding specificity and potential target genes for the farnesoid X-activated receptor. *J Biol Chem* 2000; 275(14): 10638-47.
101. Urizar NL, Dowhan DH, Moore DD. The farnesoid X-activated receptor mediates bile acid activation of phospholipid transfer protein gene expression. *J Biol Chem* 2000; 275(50): 39313-7.
102. Kast HR, Goodwin B, Tarr PT, Jones SA, Anisfeld AM, Stoltz CM, Tontonoz P, et al. Regulation of multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor, and constitutive androstane receptor. *J Biol Chem* 2002; 277(4): 2908-15.
103. Song CS, Echchgadda I, Baek BS, Ahn SC, Oh T, Roy AK, Chatterjee B. Dehydroepiandrosterone sulfotransferase gene induction by bile acid activated farnesoid X receptor. *J Biol Chem* 2001; 276(45): 42549-56.
104. Barbier O, Torra IP, Sirvent A, Claudel T, Blanquart C, Duran-Sandoval D, Kuipers F, et al. FXR induces the UGT2B4 enzyme in hepatocytes: a potential mechanism of negative feedback control of FXR activity. *Gastroenterology* 2003; 124(7): 1926-40.
105. Ananthanarayanan M, Balasubramanian N, Makishima M, Mangelsdorf DJ, Suchy FJ. Human bile salt export pump promoter is transactivated by the farnesoid X receptor/bile acid receptor. *J Biol Chem* 2001; 276(31): 28857-65.
106. Gerloff T, Geier A, Roots I, Meier PJ, Gartung C. Functional analysis of the rat bile salt export pump gene promoter. *Eur J Biochem* 2002; 269(14): 3495-503.
107. Plass JR, Mol O, Heegsma J, Geuken M, Faber KN, Jansen PL, Muller M. Farnesoid X receptor and bile salts are involved in transcriptional regulation of the gene encoding the human bile salt export pump. *Hepatology* 2002; 35(3): 589-96.
108. Kitada H, Miyata M, Nakamura T, Tozawa A, Honma W, Shimada M, Nagata K, et al. Protective role of hydroxysteroid sulfotransferase in lithocholic acid-induced liver toxicity. *J Biol Chem* 2003; 278(20): 17838-44.
109. Jung D, Podvinec M, Meyer UA, Mangelsdorf DJ, Fried M, Meier PJ, Kullak-Ublick GA. Human organic anion transporting polypeptide 8 promoter is transactivated by the farnesoid X receptor/bile acid receptor. *Gastroenterology* 2002; 122(7): 1954-66.
110. Huang L, Zhao A, Lew JL, Zhang T, Hrywna Y, Thompson JR, de PN, et al. Farnesoid X receptor activates transcription of the phospholipid pump MDR3. *J Biol Chem* 2003; 278(51): 51085-90.
111. Grober J, Zaghini I, Fujii H, Jones SA, Klierer SA, Willson TM, Ono T, et al. Identification of a bile acid-responsive element in the human ileal bile acid-binding protein gene. Involvement of the farnesoid X receptor/9-cis-retinoic acid receptor heterodimer. *J Biol Chem* 1999; 274(42): 29749-54.
112. Sinal CJ, Tohkin M, Miyata M, Ward JM, Lambert G, Gonzalez FJ. Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. *Cell* 2000; 102(6): 731-44.
113. Pircher PC, Kitto JL, Petrowski ML, Tangirala RK, Bischoff ED, Schulman IG, Westin SK. Farnesoid X receptor regulates bile acid-amino acid conjugation. *J Biol Chem* 2003; 278(30): 27703-11.
114. Echchgadda I, Song CS, Roy AK, Chatterjee B. Dehydroepiandrosterone sulfotransferase is a target for transcriptional induction by the vitamin D receptor. *Mol Pharmacol* 2004; 65(3): 720-9.
115. Denson LA, Bohan A, Bajwa HJ, Held MA, Boyer JL. Alterations in nuclear hormone receptors (NHR) are associated with changes in the expression of multidrug resistance proteins Mrp2 and Mrp3 in liver and kidney in cholestasis. *Hepatology* 2001; 34[4, Suppl 2], 367.
116. Guo GL, Lambert G, Negishi M, Ward JM, Brewer HB, Jr., Klierer SA, Gonzalez FJ, et al. Complementary roles of farnesoid X receptor, pregnane X receptor, and constitutive androstane receptor in protection against bile acid toxicity. *J Biol Chem* 2003; 278(46): 45062-71.
117. Chen F, Ananthanarayanan M, Emre S, Neimark E, Bull LN, Knisely AS, Strautnieks SS, et al. Progressive familial intrahepatic cholestasis, type 1, is associated with decreased farnesoid X receptor activity. *Gastroenterology* 2004; 126(3): 756-64.
118. Goodwin B, Jones SA, Price RR, Watson MA, McKee DD, Moore LB, Galardi C, et al. A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. *Mol Cell* 2000; 6(3): 517-26.

119. Kanaya E, Shiraki T, Jingami H. The nuclear bile acid receptor FXR is activated by PGC-1 α in a ligand-dependent manner. *Biochem J* 2004; 382(Pt 3): 913-21.
120. Lee YK, Dell H, Dowhan DH, Hadzopoulou-Cladaras M, Moore DD. The orphan nuclear receptor SHP inhibits hepatocyte nuclear factor 4 and retinoid X receptor transactivation: two mechanisms for repression. *Mol Cell Biol* 2000; 20(1): 187-95.
121. Li D, Zimmerman TL, Thevananther S, Lee HY, Kurie JM, Karpen SJ. Interleukin-1 beta-mediated suppression of RXR:RAR transactivation of the Ntcp promoter is JNK-dependent. *J Biol Chem* 2002; 277(35): 31416-22.
122. Denson LA, Sturm E, Echevarria W, Zimmerman TL, Makishima M, Mangelsdorf DJ, Karpen SJ. The orphan nuclear receptor, shp, mediates bile acid-induced inhibition of the rat bile acid transporter, ntcp. *Gastroenterology* 2001; 121(1): 140-7.
123. Zollner G, Fickert P, Silbert D, Fuchsbichler A, Stumppner C, Zatloukal K, Denk H, et al. Induction of short heterodimer partner 1 precedes downregulation of Ntcp in bile duct-ligated mice. *Am J Physiol Gastrointest Liver Physiol* 2002; 282(1): G184-G191.
124. Wang L, Lee YK, Bundman D, Han Y, Thevananther S, Kim CS, Chua SS, et al. Redundant pathways for negative feedback regulation of bile acid production. *Dev Cell* 2002; 2(6): 721-31.
125. De FE, Mitro N, Gilardi F, Caruso D, Galli G, Crestani M. Coordinated control of cholesterol catabolism to bile acids and of gluconeogenesis via a novel mechanism of transcription regulation linked to the fasted-to-fed cycle. *J Biol Chem* 2003; 278(40): 39124-32.
126. Gupta S, Stravitz RT, Dent P, Hylemon PB. Down-regulation of cholesterol 7 α -hydroxylase (CYP7A1) gene expression by bile acids in primary rat hepatocytes is mediated by the c-Jun N-terminal kinase pathway. *J Biol Chem* 2001; 276(19): 15816-22.
127. Trauner M, Arrese M, Lee H, Boyer JL, Karpen SJ. Endotoxin downregulates rat hepatic ntcp gene expression via decreased activity of critical transcription factors. *J Clin Invest* 1998; 101(10): 2092-100.
128. Denson LA, Auld KL, Schiek DS, McClure MH, Mangelsdorf DJ, Karpen SJ. Interleukin-1 β suppresses retinoid transactivation of two hepatic transporter genes involved in bile formation. *J Biol Chem* 2000; 275(12): 8835-43.
129. Wang B, Cai SR, Gao C, Sladek FM, Ponder KP. Lipopolysaccharide results in a marked decrease in hepatocyte nuclear factor 4 α in rat liver. *Hepatology* 2001; 34(5): 979-89.
130. Zhang M, Chiang JY. Transcriptional regulation of the human sterol 12 α -hydroxylase gene (CYP8B1): roles of hepatocyte nuclear factor 4 α in mediating bile acid repression. *J Biol Chem* 2001; 276(45): 41690-9.
131. Ktistaki E, Talianidis I. Modulation of hepatic gene expression by hepatocyte nuclear factor 1. *Science* 1997; 277(5322): 109-12.
132. Shih DQ, Bussen M, Sehaye E, Ananthanarayanan M, Shneider BL, Suchy FJ, Shefer S, et al. Hepatocyte nuclear factor-1 α is an essential regulator of bile acid and plasma cholesterol metabolism. *Nat Genet* 2001; 27(4): 375-82.
133. Hayhurst GP, Lee YH, Lambert G, Ward JM, Gonzalez FJ. Hepatocyte nuclear factor 4 α (nuclear receptor 2A1) is essential for maintenance of hepatic gene expression and lipid homeostasis. *Mol Cell Biol* 2001; 21(4): 1393-403.
134. Jung D, Hagenbuch B, Gresh L, Pontoglio M, Meier PJ, Kullak-Ublick GA. Characterization of the human OATP-C (SLC21A6) gene promoter and regulation of liver-specific OATP genes by hepatocyte nuclear factor 1 α . *J Biol Chem* 2001; 276(40): 37206-14.
135. Li N, Klaassen CD. Role of liver-enriched transcription factors in the down-regulation of organic anion transporting polypeptide 4 (oatp4; oatp1b2; slc21a10) by lipopolysaccharide. *Mol Pharmacol* 2004; 66(3): 694-701.
136. Chen F, Ma L, Dawson PA, Sinal CJ, Sehaye E, Gonzalez FJ, Breslow J, et al. Liver receptor homologue-1 mediates species- and cell line-specific bile acid-dependent negative feedback regulation of the apical sodium-dependent bile acid transporter. *J Biol Chem* 2003; 278(22): 19909-16.
137. Neimark E, Chen F, Li X, Shneider BL. Bile acid-induced negative feedback regulation of the human ileal bile acid transporter. *Hepatology* 2004; 40(1): 149-56.
138. Schuetz EG, Strom S, Yasuda K, Lecureux V, Assem M, Brimer C, Lamba J, et al. Disrupted bile acid homeostasis reveals an unexpected interaction among nuclear hormone receptors, transporters, and cytochrome P450. *J Biol Chem* 2001; 276(42): 39411-8.
139. Jones SA, Moore LB, Shenk JL, Wisely GB, Hamilton GA, McKee DD, Tomkinson NC, et al. The pregnane X receptor: a promiscuous xenobiotic receptor that has diverged during evolution. *Mol Endocrinol* 2000; 14(1): 27-39.
140. Watkins RE, Wisely GB, Moore LB, Collins JL, Lambert MH, Williams SP, Willson TM, et al. The human nuclear xenobiotic receptor PXR: structural determinants of directed promiscuity. *Science* 2001; 292(5525): 2329-33.
141. Lehmann JM, McKee DD, Watson MA, Willson TM, Moore JT, Kliewer SA. The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J Clin Invest* 1998; 102(5): 1016-23.
142. Moore LB, Parks DJ, Jones SA, Bledsoe RK, Consler TG, Stimmel JB, Goodwin B, et al. Orphan nuclear receptors constitutive androstane receptor and pregnane X receptor share xenobiotic and steroid ligands. *J Biol Chem* 2000; 275(20): 15122-7.
143. Landes N, Pfluger P, Kluth D, Birringer M, Ruhl R, Bol GF, Glatt H, et al. Vitamin E activates gene expression via the pregnane X receptor. *Biochem Pharmacol* 2003; 65(2): 269-73.
144. Moore LB, Goodwin B, Jones SA, Wisely GB, Serabjit-Singh CJ, Willson TM, Collins JL, et al. St. John's wort induces hepatic drug metabolism through activation of the pregnane X receptor. *Proc Natl Acad Sci USA* 2000; 97(13): 7500-2.
145. Zhang H, LeCulysse E, Liu L, Hu M, Matoney L, Zhu W, Yan B. Rat pregnane X receptor: molecular cloning, tissue distribution, and xenobiotic regulation. *Arch Biochem Biophys* 1999; 368(1): 14-22.
146. Geick A, Eichelbaum M, Burk O. Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampin. *J Biol Chem* 2001; 276(18): 14581-7.
147. Synold TW, Dussault I, Forman BM. The orphan nuclear receptor SXR coordinately regulates drug metabolism and efflux. *Nat Med* 2001; 7(5): 584-90.
148. Rosenfeld JM, Vargas R, Jr., Xie W, Evans RM. Genetic profiling defines the xenobiotic gene network controlled by the nuclear receptor pregnane X receptor. *Mol Endocrinol* 2003; 17(7): 1268-82.
149. Teng S, Jekerle V, Piquette-Miller M. Induction of ABC3 (MRP3) by pregnane X receptor activators. *Drug Metab Dispos* 2003; 31(11): 1296-9.
150. Wagner M, Halilbasic E, Zollner G, Fickert P, Gumhold J, Silbert D, Fuchsbichler A, et al. Xenobiotic nuclear receptor (PXR, CAR) agonists coordinately stimulate a spectrum of detoxification enzymes and transport systems involved in the adaptive response to cholestasis *in vivo*. *Hepatology* 2004; 40, Suppl. 1.
151. Li T, Chiang JY. Mechanism of rifampicin and pregnane X receptor inhibition of human cholesterol 7 α -hydroxylase gene transcription. *Am J Physiol Gastrointest Liver Physiol* 2005; 288(1): G74-G84.
152. Bhalla S, Ozalp C, Fang S, Xiang L, Kemper JK. Ligand-activated pregnane X receptor interferes with HNF-4 signaling by targeting a common coactivator PGC-1 α . Functional implications in hepatic cholesterol and glucose metabolism. *J Biol Chem* 2004; 279(43): 45139-47.
153. Sonoda J, Xie W, Rosenfeld JM, Barwick JL, Guzelian PS, Evans RM. Regulation of a xenobiotic sulfonation cascade by nuclear pregnane X receptor (PXR). *Proc Natl Acad Sci USA* 2002; 99(21): 13801-6.
154. Chen C, Staudinger JL, Klaassen CD. Nuclear receptor, pregnane X receptor, is required for induction of UDP-glucuronosyltransferases in mouse liver by pregnenolone-16 α -carbonitrile. *Drug Metab Dispos* 2003; 31(7): 908-15.
155. Maglich JM, Stoltz CM, Goodwin B, Hawkins-Brown D, Moore JT, Kliewer SA. Nuclear pregnane x receptor and constitutive an-

- drostane receptor regulate overlapping but distinct sets of genes involved in xenobiotic detoxification. *Mol Pharmacol* 2002; 62(3): 638-46.
156. Frank C, Makkonen H, Dunlop TW, Matilainen M, Vaisanen S, Carlberg C. Identification of pregnane X receptor binding sites in the regulatory regions of genes involved in bile acid homeostasis. *J Mol Biol* 2005; 346(2): 505-19.
157. Ourlin JC, Lasserre F, Pineau T, Fabre JM, Sa-Cunha A, Maurel P, Vilarem MJ, et al. The small heterodimer partner interacts with the pregnane X receptor and represses its transcriptional activity. *Mol Endocrinol* 2003; 17(9): 1693-703.
158. Goodwin B, Moore JT. CAR: detailing new models. *Trends Pharmacol Sci* 2004; 25(8): 437-41.
159. Frank C, Gonzalez MM, Oinonen C, Dunlop TW, Carlberg C. Characterization of DNA complexes formed by the nuclear receptor constitutive androstane receptor. *J Biol Chem* 2003; 278(44): 43299-310.
160. Baes M, Gulick T, Choi HS, Martinoli MG, Simha D, Moore DD. A new orphan member of the nuclear hormone receptor superfamily that interacts with a subset of retinoic acid response elements. *Mol Cell Biol* 1994; 14(3): 1544-52.
161. Choi HS, Chung M, Tzameli I, Simha D, Lee YK, Seol W, Moore DD. Differential transactivation by two isoforms of the orphan nuclear hormone receptor CAR. *J Biol Chem* 1997; 272(38): 23565-71.
162. Xie W, Barwick JL, Simon CM, Pierce AM, Safe S, Blumberg B, Guzelian PS, et al. Reciprocal activation of xenobiotic response genes by nuclear receptors SXR/PXR and CAR. *Genes Dev* 2000; 14(23): 3014-23.
163. Zhang J, Huang W, Qatanani M, Evans RM, Moore DD. The constitutive androstane receptor and pregnane X receptor function coordinately to prevent bile acid-induced hepatotoxicity. *J Biol Chem* 2004; in press.
164. Saini SP, Sonoda J, Xu L, Toma D, Uppal H, Mu Y, Ren S, et al. A novel constitutive androstane receptor-mediated and CYP3A-independent pathway of bile acid detoxification. *Mol Pharmacol* 2004; 65(2): 292-300.
165. Huang W, Zhang J, Moore DD. A traditional herbal medicine enhances bilirubin clearance by activating the nuclear receptor CAR. *J Clin Invest* 2004; 113(1): 137-43.
166. Bae Y, Kemper JK, Kemper B. Repression of CAR-mediated transactivation of CYP2B genes by the orphan nuclear receptor, short heterodimer partner (SHP). *DNA Cell Biol* 2004; 23(2): 81-91.
167. Gascon-Barre M, Demers C, Mirshahi A, Neron S, Zalzal S, Nanci A. The normal liver harbors the vitamin D nuclear receptor in nonparenchymal and biliary epithelial cells. *Hepatology* 2003; 37(5): 1034-42.
168. Chen X, Pang SK, Chen F, Shneider BL. 1 alpha,25-dihydroxyvitamin D-3 up-regulates the rat apical sodium-dependent bile acid transporter (ASBT) via a specific vitamin D response element (VDRE) in the promoter region of ASBT gene. *Hepatology* 2004; 40[4, Suppl 2], 519A.
169. Honjo Y, Kobayashi Y, Sasaki S, Misawa H, Nakamura H. Inhibition of farnesoid X receptor-induced BSEP gene expression by ligand-activated vitamin D receptor. *Hepatology* 2004; 40[4, Suppl. 1].
170. Chen WS, Denk GU, Denson LA, Wang L, Soroka C, Boyer JL. Release of transcriptional repression from RXRa and RARa results in up-regulation of Mrp3 (the multidrug resistance-associated protein 3) in obstructive cholestasis. *Hepatology* 2004; 40[4, Suppl 1].
171. Inokuchi A, Hinoshita E, Iwamoto Y, Kohno K, Kuwano M, Uchiyama T. Enhanced expression of the human multidrug resistance protein 3 by bile salt in human enterocytes. A transcriptional control of a plausible bile acid transporter. *J Biol Chem* 2001; 276(50): 46822-9.
172. del Castillo-Olivares A, Gil G. Alpha 1-fetoprotein transcription factor is required for the expression of sterol 12alpha-hydroxylase, the specific enzyme for cholic acid synthesis. Potential role in the bile acid-mediated regulation of gene transcription. *J Biol Chem* 2000; 275(23): 17793-9.
173. Bohan A, Chen WS, Denson LA, Held MA, Boyer JL. Tumor necrosis factor alpha-dependent up-regulation of Lrh-1 and Mrp3(Abcc3) reduces liver injury in obstructive cholestasis. *J Biol Chem* 2003; 278(38): 36688-98.
174. Yang Y, Zhang M, Eggertsen G, Chiang JY. On the mechanism of bile acid inhibition of rat sterol 12alpha-hydroxylase gene (CYP8B1) transcription: roles of alpha-fetoprotein transcription factor and hepatocyte nuclear factor 4alpha. *Biochim Biophys Acta* 2002; 1583(1): 63-73.
175. Chianale J, Vollrath V, Wielandt AM, Amigo L, Rigotti A, Nervi F, Gonzalez S, et al. Fibrates induce mdr2 gene expression and biliary phospholipid secretion in the mouse. *Biochem J* 1996; 314 (Pt 3): 781-6.
176. Jung D, Fried M, Kullak-Ublick GA. Human apical sodium-dependent bile salt transporter gene (SLC10A2) is regulated by the peroxisome proliferator-activated receptor alpha. *J Biol Chem* 2002; 277(34): 30559-66.
177. Hunt MC, Yang YZ, Eggertsen G, Carneheim CM, Gafvels M, Einarsson C, Alexson SE. The peroxisome proliferator-activated receptor alpha (PPARalpha) regulates bile acid biosynthesis. *J Biol Chem* 2000; 275(37): 28947-53.
178. Patel DD, Knight BL, Soutar AK, Gibbons GF, Wade DP. The effect of peroxisome-proliferator-activated receptor-alpha on the activity of the cholesterol 7 alpha-hydroxylase gene. *Biochem J* 2000; 351 Pt 3: 747-53.
179. Barbier O, Villeneuve L, Bocher V, Fontaine C, Torra IP, Duhem C, Kosykh V, et al. The UDP-glucuronosyltransferase 1A9 enzyme is a peroxisome proliferator-activated receptor alpha and gamma target gene. *J Biol Chem* 2003; 278(16): 13975-83.
180. Fang HL, Strom SC, Cai H, Falany CN, Kocarek TA, Runge-Morris M. Regulation of Human Hepatic Hydroxysteroid Sulfotransferase Gene Expression by the Peroxisome Proliferator Activated Receptor Alpha Transcription Factor. *Mol Pharmacol* 2005.
181. Eloranta JJ, Jung D, Kullak-Ublick G. The human Na plus taurocholate cotransporting polypeptide gene (NTCP, SLC10A1) is activated by the glucocorticoid receptor and its coactivator PGC-1 and suppressed by the small heterodimer partner SHP. *Hepatology* 2004; 40[4, Suppl 1], 519A.
182. Alvaro D, Gigliozi A, Marucci L, Alpini G, Barbaro B, Monterubbianesi R, Minetola L, et al. Corticosteroids modulate the secretory processes of the rat intrahepatic biliary epithelium. *Gastroenterology* 2002; 122(4): 1058-69.
183. Pascucci JM, Busson-Le CM, Maurel P, Vilarem MJ. Transcriptional analysis of the orphan nuclear receptor constitutive androstane receptor (NR1I3) gene promoter: identification of a distal glucocorticoid response element. *Mol Endocrinol* 2003; 17(1): 42-55.
184. Miura T, Ouchida R, Yoshikawa N, Okamoto K, Makino Y, Nakamura T, Morimoto C, et al. Functional modulation of the glucocorticoid receptor and suppression of NF-kappaB-dependent transcription by ursodeoxycholic acid. *J Biol Chem* 2001; 276(50): 47371-8.
185. Trauner M, Meier PJ, Boyer JL. Molecular regulation of hepatocellular transport systems in cholestasis. *J Hepatol* 1999; 31(1): 165-78.
186. Trauner M, Boyer JL. Cholestatic syndromes. *Curr Opin Gastroenterol* 2004; 20(3): 220-30.
187. Bull LN, Carlton VE, Stricker NL, Baharloo S, DeYoung JA, Freimer NB, Magid MS, et al. Genetic and morphological findings in progressive familial intrahepatic cholestasis (Byler disease [PFIC-1] and Byler syndrome): evidence for heterogeneity. *Hepatology* 1997; 26(1): 155-64.
188. Clayton RJ, Iber FL, Ruebner BH, McKusick VA. Byler disease. Fatal familial intrahepatic cholestasis in an Amish kindred. *Am J Dis Child* 1969; 117(1): 112-24.
189. Eppens EF, van Mil SW, de Vree JM, Mok KS, Juijn JA, Oude Elferink RP, Berger R, et al. FIC1, the protein affected in two forms of hereditary cholestasis, is localized in the cholangiocyte and the canalicular membrane of the hepatocyte. *J Hepatol* 2001; 35(4): 436-43.
190. Ujhazy P, Ortiz D, Misra S, Li S, Moseley J, Jones H, Arias IM. Familial intrahepatic cholestasis 1: studies of localization and function. *Hepatology* 2001; 34(4 Pt 1): 768-75.
191. Bull LN, van Eijk MJ, Pawlikowska L, DeYoung JA, Juijn JA, Liao M, Klomp LW, et al. A gene encoding a P-type ATPase mutated in two forms of hereditary cholestasis. *Nat Genet* 1998; 18(3): 219-24.

192. Stieger B. FIC1: another bile salt carrier within the enterohepatic circulation? *J Hepatol* 2001; 35(4): 522-4.
193. Harris MJ, Kagawa T, Dawson PA, Arias IM. The effect of FIC1 expression on taurocholate transport by BSEP and IBAT in transfected MDCKII cells. *Hepatology* 2002; 36[4; Suppl 2], 309A.
194. Pawlikowska L, Groen A, Eppens EF, Kunne C, Ottenhoff R, Looije N, Knisely AS, et al. A mouse genetic model for familial cholestasis caused by ATP8B1 mutations reveals perturbed bile salt homeostasis but no impairment in bile secretion. *Hum Mol Genet* 2004; 13(8): 881-92.
195. Kok T, Hulzebos CV, Wolters H, Havinga R, Agellon LB, Stellaard F, Shan B, et al. Enterohepatic circulation of bile salts in farnesoid X receptor-deficient mice: efficient intestinal bile salt absorption in the absence of ileal bile acid-binding protein. *J Biol Chem* 2003; 278(43): 41930-7.
196. van Mil SW, van der Woerd WL, van der BG, Sturm E, Jansen PL, Bull LN, van dB I, et al. Benign recurrent intrahepatic cholestasis type 2 is caused by mutations in ABCB11. *Gastroenterology* 2004; 127(2): 379-84.
197. Strautnieks SS, Bull LN, Knisely AS, Kocoshis SA, Dahl N, Arnell H, Sokal E, et al. A gene encoding a liver-specific ABC transporter is mutated in progressive familial intrahepatic cholestasis. *Nat Genet* 1998; 20(3): 233-8.
198. Setchell KD, Rodrigues CM, Clerici C, Solinas A, Morelli A, Gartung C, Boyer J. Bile acid concentrations in human and rat liver tissue and in hepatocyte nuclei. *Gastroenterology* 1997; 112(1): 226-35.
199. de Vree JM, Jacquemin E, Sturm E, Cresteil D, Bosma PJ, Aten J, Deleuze JF, et al. Mutations in the MDR3 gene cause progressive familial intrahepatic cholestasis. *Proc Natl Acad Sci USA* 1998; 95(1): 282-7.
200. Jansen PL, Muller M. The molecular genetics of familial intrahepatic cholestasis. *Gut* 2000; 47(1): 1-5.
201. Jacquemin E, de Vree JM, Cresteil D, Sokal EM, Sturm E, Dumont M, Scheffer GL, et al. The wide spectrum of multidrug resistance 3 deficiency: from neonatal cholestasis to cirrhosis of adulthood. *Gastroenterology* 2001; 120(6): 1448-58.
202. Rosmorduc O, Hermelin B, Poupon R. MDR3 gene defect in adults with symptomatic intrahepatic and gallbladder cholesterol cholelithiasis. *Gastroenterology* 2001; 120(6): 1459-67.
203. Arrese M, Accatino L. Is intrahepatic cholestasis of pregnancy an MDR3-related disease? *Gastroenterology* 2003; 125(6): 1922-3.
204. Fickert P, Zollner G, Fuchsbichler A, Stumptner C, Weiglein AH, Lammert F, Marschall HU, et al. Ursodeoxycholic acid aggravates bile infarcts in bile duct-ligated and Mdr2 knockout mice via disruption of cholangioles. *Gastroenterology* 2002; 123(4): 1238-51.
205. Lammert F, Wang DQ, Hillebrandt S, Geier A, Fickert P, Trauner M, Matern S, et al. Spontaneous cholecysto- and hepatolithiasis in Mdr2^{-/-} mice: a model for low phospholipid-associated cholelithiasis. *Hepatology* 2004; 39(1): 117-28.
206. Dumoulin FL, Reichel C, Sauerbruch T, Spengler U. Semiquantitation of intrahepatic MDR3 mRNA levels by reverse transcription/competitive polymerase chain reaction. *J Hepatol* 1997; 26(4): 852-6.
207. Zollner G, Fickert P, Silbert D, Fuchsbichler A, Marschall HU, Zatloukal K, Denk H, et al. Adaptive changes in hepatobiliary transporter expression in primary biliary cirrhosis. *J Hepatol* 2003; 38(6): 717-27.
208. Pauli-Magnus C, Kerb R, Fattinger K, Lang T, Anwald B, Kullak-Ublick GA, Beuers U, et al. BSEP and MDR3 haplotype structure in healthy Caucasians, primary biliary cirrhosis and primary sclerosing cholangitis. *Hepatology* 2004; 39(3): 779-91.
209. Paulusma CC, Kool M, Bosma PJ, Scheffer GL, ter Borg F, Scheper RJ, Tytgat GN, et al. A mutation in the human canalicular multispecific organic anion transporter gene causes the Dubin-Johnson syndrome. *Hepatology* 1997; 25(6): 1539-42.
210. Kartenbeck J, Leuschner U, Mayer R, Keppler D. Absence of the canalicular isoform of the MRP gene-encoded conjugate export pump from the hepatocytes in Dubin-Johnson syndrome. *Hepatology* 1996; 23(5): 1061-6.
211. Wada M, Toh S, Taniguchi K, Nakamura T, Uchiyumi T, Kohno K, Yoshida I, et al. Mutations in the canalicular multispecific organic anion transporter (cMOAT) gene, a novel ABC transporter, in patients with hyperbilirubinemia II/Dubin-Johnson syndrome. *Hum Mol Genet* 1998; 7(2): 203-7.
212. Konig J, Rost D, Cui Y, Keppler D. Characterization of the human multidrug resistance protein isoform MRP3 localized to the basolateral hepatocyte membrane. *Hepatology* 1999; 29(4): 1156-63.
213. Jansen PL, Peters WH, Lamers WH. Hereditary chronic conjugated hyperbilirubinemia in mutant rats caused by defective hepatic anion transport. *Hepatology* 1985; 5(4): 573-9.
214. Kuipers F, Enserink M, Havinga R, van der Steen AB, Hardonk MJ, Fevery J, Vonk RJ. Separate transport systems for biliary secretion of sulfated and unsulfated bile acids in the rat. *J Clin Invest* 1988; 81(5): 1593-9.
215. Ito K, Suzuki H, Hirohashi T, Kume K, Shimizu T, Sugiyama Y. Molecular cloning of canalicular multispecific organic anion transporter defective in EHBR. *Am J Physiol* 1997; 272(1 Pt 1): G16-G22.
216. Tirona RG, Kim RB. Pharmacogenomics of organic anion-transporting polypeptides (OATP). *Adv Drug Deliv Rev* 2002; 54(10): 1343-52.
217. Zelcer N, van de Wetering K, de Waart DR, Kunne C, Elferink RO, Borst P. MRP3 knockout mice have normal bile salt homeostasis but altered bilirubin disposition. *Hepatology* 2004; 40[4, Suppl 1], 489A.
218. Levy C, Lindor KD. Drug-induced cholestasis. *Clin Liver Dis* 2003; 7(2): 311-30.
219. Riely CA, Bacq Y. Intrahepatic cholestasis of pregnancy. *Clin Liver Dis* 2004; 8(1): 167-76.
220. Li MK, Crawford JM. The pathology of cholestasis. *Semin Liver Dis* 2004; 24(1): 21-42.
221. Glantz A, Marschall HU, Mattsson LA. Intrahepatic cholestasis of pregnancy: Relationships between bile acid levels and fetal complication rates. *Hepatology* 2004; 40(2): 467-74.
222. Roman ID, Fernandez-Moreno MD, Fueyo JA, Roma MG, Coleman R. Cyclosporin A induced internalization of the bile salt export pump in isolated rat hepatocyte couplets. *Toxicol Sci* 2003; 71(2): 276-81.
223. Crocenzi FA, Mottino AD, Cao J, Veggi LM, Pozzi EJ, Vore M, Coleman R, et al. Estradiol-17beta-D-glucuronide induces endocytic internalization of Bsep in rats. *Am J Physiol Gastrointest Liver Physiol* 2003; 285(2): G449-G459.
224. Mottino AD, Cao J, Veggi LM, Crocenzi F, Roma MG, Vore M. Altered localization and activity of canalicular Mrp2 in estradiol-17beta-D-glucuronide-induced cholestasis. *Hepatology* 2002; 35(6): 1409-19.
225. Fattinger K, Funk C, Pantze M, Weber C, Reichen J, Stieger B, Meier PJ. The endothelin antagonist bosentan inhibits the canalicular bile salt export pump: a potential mechanism for hepatic adverse reactions. *Clin Pharmacol Ther* 2001; 69(4): 223-31.
226. Fouassier L, Kinnman N, Lefevre G, Lasnier E, Rey C, Poupon R, Elferink RP, et al. Contribution of mrp2 in alterations of canalicular bile formation by the endothelin antagonist bosentan. *J Hepatol* 2002; 37(2): 184-91.
227. Pauli-Magnus C, Lang T, Meier Y, Zodan-Marin T, Jung D, Breymann C, Zimmermann R, et al. Sequence analysis of bile salt export pump (ABCB11) and multidrug resistance p-glycoprotein 3 (ABCB4, MDR3) in patients with intrahepatic cholestasis of pregnancy. *Pharmacogenetics* 2004; 14(2): 91-102.
228. Vore M. Estrogen cholestasis. Membranes, metabolites, or receptors? *Gastroenterology* 1987; 93(3): 643-9.
229. Reyes H, Simon FR. Intrahepatic cholestasis of pregnancy: an estrogen-related disease. *Semin Liver Dis* 1993; 13(3): 289-301.
230. Lee JM, Trauner M, Soroka CJ, Stieger B, Meier PJ, Boyer JL. Expression of the bile salt export pump is maintained after chronic cholestasis in the rat. *Gastroenterology* 2000; 118(1): 163-72.
231. Bossard R, Stieger B, O'Neill B, Fricker G, Meier PJ. Ethinylestradiol treatment induces multiple canalicular membrane transport alterations in rat liver. *J Clin Invest* 1993; 91(6): 2714-20.
232. Simon FR, Fortune J, Iwahashi M, Gartung C, Wolkoff A, Sutherland E. Ethinyl estradiol cholestasis involves alterations in expression of liver sinusoidal transporters. *Am J Physiol* 1996; 271(6 Pt 1): G1043-G1052.
233. Lai K, Harnish DC, Evans MJ. Estrogen receptor alpha regulates expression of the orphan receptor small heterodimer partner. *J Biol Chem* 2003; 278(38): 36418-29.

234. Arrese M, Trauner M, Ananthanarayanan M, Pizarro M, Solis N, Accatino L, Soroka C, et al. Down-regulation of the Na⁺/taurocholate cotransporting polypeptide during pregnancy in the rat. *J Hepatol* 2003; 38(2): 148-55.
235. Wagner M, Fickert P, Zollner G, Silbert D, Gumhold J, Fuchsbichler A, Zatloukal K, et al. Hepatic and renal ABC transporter expression in mice depends on gender and changes in pregnancy. *Gastroenterology* 2004; 26[4, Suppl 2], A130.
236. Cao J, Huang L, Liu Y, Hoffman T, Stieger B, Meier PJ, Vore M. Differential regulation of hepatic bile salt and organic anion transporters in pregnant and postpartum rats and the role of prolactin. *Hepatology* 2001; 33(1): 140-7.
237. Cao J, Stieger B, Meier PJ, Vore M. Expression of rat hepatic multidrug resistance-associated proteins and organic anion transporters in pregnancy. *Am J Physiol Gastrointest Liver Physiol* 2002; 283(3): G757-G766.
238. Masuyama H, Hiramatsu Y, Mizutani Y, Inoshita H, Kudo T. The expression of pregnane X receptor and its target gene, cytochrome P450 3A1, in perinatal mouse. *Mol Cell Endocrinol* 2001; 172(1-2): 47-56.
239. Lamba V, Lamba J, Yasuda K, Strom S, Davila J, Hancock ML, Fackenthal JD, et al. Hepatic CYP2B6 expression: gender and ethnic differences and relationship to CYP2B6 genotype and CAR (constitutive androstane receptor) expression. *J Pharmacol Exp Ther* 2003; 307(3): 906-22.
240. Wang R, Lam P, Liu L, Forrest D, Yousef IM, Mignault D, Phillips MJ, et al. Severe cholestasis induced by cholic acid feeding in knockout mice of sister of P-glycoprotein. *Hepatology* 2003; 38(6): 1489-99.
241. Trauner M, Fickert P, Stauber RE. Inflammation-induced cholestasis. *J Gastroenterol Hepatol* 1999; 14(10): 946-59.
242. Zollner G, Fickert P, Zenz R, Fuchsbichler A, Stumptner C, Kenner L, Ferenci P, et al. Hepatobiliary transporter expression in percutaneous liver biopsies of patients with cholestatic liver diseases. *Hepatology* 2001; 33(3): 633-46.
243. Bolder U, Ton-Nu HT, Scheingart CD, Frick E, Hofmann AF. Hepatocyte transport of bile acids and organic anions in endotoxemic rats: impaired uptake and secretion. *Gastroenterology* 1997; 112(1): 214-25.
244. Roelofs H, Schoemaker B, Bakker C, Ottenhoff R, Jansen PL, Elferink RP. Impaired hepatocanalicular organic anion transport in endotoxemic rats. *Am J Physiol* 1995; 269(3 Pt 1): G427-G434.
245. Whiting JF, Green RM, Rosenbluth AB, Gollan JL. Tumor necrosis factor- α decreases hepatocyte bile salt uptake and mediates endotoxin-induced cholestasis. *Hepatology* 1995; 22(4 Pt 1): 1273-8.
246. Moseley RH, Wang W, Takeda H, Lown K, Shick L, Ananthanarayanan M, Suchy FJ. Effect of endotoxin on bile acid transport in rat liver: a potential model for sepsis-associated cholestasis. *Am J Physiol* 1996; 271(1 Pt 1): G137-G146.
247. Geier A, Dietrich CG, Voigt S, Kim SK, Gerloff T, Kullak-Ublick GA, Lorenzen J, et al. Effects of proinflammatory cytokines on rat organic anion transporters during toxic liver injury and cholestasis. *Hepatology* 2003; 38(2): 345-54.
248. Green RM, Beier D, Gollan JL. Regulation of hepatocyte bile salt transporters by endotoxin and inflammatory cytokines in rodents. *Gastroenterology* 1996; 111(1): 193-8.
249. Karpen SJ, Sun AQ, Kudish B, Hagenbuch B, Meier PJ, Ananthanarayanan M, Suchy FJ. Multiple factors regulate the rat liver basolateral sodium-dependent bile acid cotransporter gene promoter. *J Biol Chem* 1996; 271(25): 15211-21.
250. Beigneux AP, Moser AH, Shigenaga JK, Grunfeld C, Feingold KR. The acute phase response is associated with retinoid X receptor repression in rodent liver. *J Biol Chem* 2000; 275(21): 16390-9.
251. Sturm E, Havinga R, Baller JF, Wolters H, van RN, Kamps JA, Verkade HJ, et al. Kupffer cell depletion with liposomal clodronate prevents suppression of Ntcp expression in endotoxin-treated rats. *J Hepatol* 2005; 42(1): 102-9.
252. Geier A, Zollner G, Dietrich CG, Wagner M, Fickert P, Denk H, van RN, et al. Cytokine-independent repression of rodent Ntcp in obstructive cholestasis. *Hepatology* 2005; 41(3): 470-7.
253. Memon RA, Moser AH, Shigenaga JK, Grunfeld C, Feingold KR. *In vivo* and *in vitro* regulation of sterol 27-hydroxylase in the liver during the acute phase response. potential role of hepatocyte nuclear factor-1. *J Biol Chem* 2001; 276(32): 30118-26.
254. Ros JE, Schuetz JD, Geuken M, Streetz K, Moshage H, Kuipers F, Manns MP, et al. Induction of Mdr1b expression by tumor necrosis factor- α in rat liver cells is independent of p53 but requires NF- κ B signaling. *Hepatology* 2001; 33(6): 1425-31.
255. Assenat E, Gerbal-Chaloin S, Larrey D, Saric J, Fabre JM, Maurel P, Vilarem MJ, et al. Interleukin 1 β inhibits CAR-induced expression of hepatic genes involved in drug and bilirubin clearance. *Hepatology* 2004; 40(4): 951-60.
256. Trauner M, Arrese M, Soroka CJ, Ananthanarayanan M, Koepfel TA, Schlosser SF, Suchy FJ, et al. The rat canalicular conjugate export pump (Mrp2) is down-regulated in intrahepatic and obstructive cholestasis. *Gastroenterology* 1997; 113(1): 255-64.
257. Kubitz R, Wettstein M, Warskulat U, Haussinger D. Regulation of the multidrug resistance protein 2 in the rat liver by lipopolysaccharide and dexamethasone. *Gastroenterology* 1999; 116(2): 401-10.
258. Kim MS, Shigenaga J, Moser A, Feingold K, Grunfeld C. Repression of farnesoid X receptor during the acute phase response. *J Biol Chem* 2003; 278(11): 8988-95.
259. Beigneux AP, Moser AH, Shigenaga JK, Grunfeld C, Feingold KR. Reduction in cytochrome P-450 enzyme expression is associated with repression of CAR (constitutive androstane receptor) and PXR (pregnane X receptor) in mouse liver during the acute phase response. *Biochem Biophys Res Commun* 2002; 293(1): 145-9.
260. Fang C, Yoon S, Tindberg N, Jarvelainen HA, Lindros KO, Ingelman-Sundberg M. Hepatic expression of multiple acute phase proteins and down-regulation of nuclear receptors after acute endotoxin exposure. *Biochem Pharmacol* 2004; 67(7): 1389-97.
261. Teng S, Piquette-Miller M. The involvement of the pregnane X receptor in hepatic gene regulation during inflammation in mice. *J Pharmacol Exp Ther* 2005; 312(2): 841-8.
262. Donner MG, Warskulat U, Saha N, Haussinger D. Enhanced expression of basolateral multidrug resistance protein isoforms Mrp3 and Mrp5 in rat liver by LPS. *Biol Chem* 2004; 385(3-4): 331-9.
263. Cherrington NJ, Slitt AL, Li N, Klaassen CD. Lipopolysaccharide-mediated regulation of hepatic transporter mRNA levels in rats. *Drug Metab Dispos* 2004; 32(7): 734-41.
264. Hartmann G, Cheung AK, Piquette-Miller M. Inflammatory cytokines, but not bile acids, regulate expression of murine hepatic anion transporters in endotoxemia. *J Pharmacol Exp Ther* 2002; 303(1): 273-81.
265. Shneider BL, Fox VL, Schwarz KB, Watson CL, Ananthanarayanan M, Thevananthar S, Christie DM, et al. Hepatic basolateral sodium-dependent-bile acid transporter expression in two unusual cases of hypercholanemia and in extrahepatic biliary atresia. *Hepatology* 1997; 25(5): 1176-83.
266. Kogan D, Ananthanarayanan M, Emre S. The bile salt export pump (BSEP/SPGP) is not down-regulated in human cholestasis associated with extrahepatic biliary atresia. *Hepatology* 1999; 30, 468A.
267. Shoda J, Kano M, Oda K, Kamiya J, Nimura Y, Suzuki H, Sugiyama Y, et al. The expression levels of plasma membrane transporters in the cholestatic liver of patients undergoing biliary drainage and their association with the impairment of biliary secretory function. *Am J Gastroenterol* 2001; 96(12): 3368-78.
268. Oswald M, Kullak-Ublick GA, Paumgartner G, Beuers U. Expression of hepatic transporters OATP-C and MRP2 in primary sclerosing cholangitis. *Liver* 2001; 21(4): 247-53.
269. Gartung C, Ananthanarayanan M, Rahman MA, Schuele S, Nundy S, Soroka CJ, Stolz A, et al. Down-regulation of expression and function of the rat liver Na⁺/bile acid cotransporter in extrahepatic cholestasis. *Gastroenterology* 1996; 110(1): 199-209.
270. Dumont M, Jacquemin E, D'Hont C, Descout C, Cresteil D, Haouzi D, Desrochers M, et al. Expression of the liver Na⁺-independent organic anion transporting polypeptide (oatp-1) in rats with bile duct ligation. *J Hepatol* 1997; 27(6): 1051-6.
271. Wagner M, Fickert P, Zollner G, Fuchsbichler A, Silbert D, Tsybrovskyy O, Zatloukal K, et al. Role of farnesoid X receptor

- in determining hepatic ABC transporter expression and liver injury in bile duct-ligated mice. *Gastroenterology* 2003; 125(3): 825-38.
272. Donner MG, Keppler D. Up-regulation of basolateral multidrug resistance protein 3 (Mrp3) in cholestatic rat liver. *Hepatology* 2001; 34(2): 351-9.
 273. Denk GU, Soroka CJ, Takeyama Y, Chen WS, Schuetz JD, Boyer JL. Multidrug resistance-associated protein 4 is up-regulated in liver but down-regulated in kidney in obstructive cholestasis in the rat. *J Hepatol* 2004; 40(4): 585-91.
 274. Sewnath ME, Van Der PT, Ten Kate FJ, Van Noorden CJ, Gouma DJ. Interleukin-1 receptor type I gene-deficient bile duct-ligated mice are partially protected against endotoxin. *Hepatology* 2002; 35(1): 149-58.
 275. Plebani M, Panozzo MP, Basso D, De PM, Biasin R, Infantolino D. Cytokines and the progression of liver damage in experimental bile duct ligation. *Clin Exp Pharmacol Physiol* 1999; 26(4): 358-63.
 276. Liu TZ, Lee KT, Chern CL, Cheng JT, Stern A, Tsai LY. Free radical-triggered hepatic injury of experimental obstructive jaundice of rats involves overproduction of proinflammatory cytokines and enhanced activation of nuclear factor kappaB. *Ann Clin Lab Sci* 2001; 31(4): 383-90.
 277. Denson LA, Bohan A, Held MA, Boyer JL. Organ-specific alterations in RAR alpha:RXR alpha abundance regulate rat Mrp2 (Abcc2) expression in obstructive cholestasis. *Gastroenterology* 2002; 123(2): 599-607.
 278. Fickert P, Fuchsbichler A, Wagner M, Zollner G, Kaser A, Tilg H, Krause R, et al. Regurgitation of bile acids from leaky bile ducts causes sclerosing cholangitis in Mdr2 (Abcb4) knockout mice. *Gastroenterology* 2004; 127(1): 261-74.
 279. Wagner M, Fickert P, Zollner G, Fuchsbichler A, Gumhold J, Silbert D, Zatloukal K et al. Mdr2-/- Mice with chronic cholestasis due to sclerosing cholangitis lack adaptive regulation of ABC transporters and bile acid synthetic enzymes. *Gastroenterology* 2004; [Suppl 1].
 280. Chisholm JW, Dolphin PJ. Abnormal lipoproteins in the ANIT-treated rat: a transient and reversible animal model of intrahepatic cholestasis. *J Lipid Res* 1996; 37(5): 1086-98.
 281. Desmet VJ, Krstulovic B, Van DB. Histochemical study of rat liver in alpha-naphthyl isothiocyanate (ANIT) induced cholestasis. *Am J Pathol* 1968; 52(2): 401-21.
 282. Liu Y, Binz J, Numerick MJ, Dennis S, Luo G, Desai B, MacKenzie KI, et al. Hepatoprotection by the farnesoid X receptor agonist GW4064 in rat models of intra- and extrahepatic cholestasis. *J Clin Invest* 2003; 112(11): 1678-87.
 283. Paumgartner G, Beuers U. Ursodeoxycholic acid in cholestatic liver disease: mechanisms of action and therapeutic use revisited. *Hepatology* 2002; 36(3): 525-31.
 284. Paumgartner G, Beuers U. Mechanisms of action and therapeutic efficacy of ursodeoxycholic acid in cholestatic liver disease. *Clin Liver Dis* 2004; 8(1): 67-81, vi.
 285. Gerk PM, Vore M. Tauroursodeoxycholate and taurocholate are transported by human MRP2 in the presence of certain MRP2 substrates. *Hepatology* 2004; 40, Suppl. 1.
 286. Trauner M, Graziadei IW. Review article: mechanisms of action and therapeutic applications of ursodeoxycholic acid in chronic liver diseases. *Aliment Pharmacol Ther* 1999; 13(8): 979-96.
 287. Fickert P, Zollner G, Fuchsbichler A, Stumptner C, Pojer C, Zenz R, Lammert F, et al. Effects of ursodeoxycholic and cholic acid feeding on hepatocellular transporter expression in mouse liver. *Gastroenterology* 2001; 121(1): 170-83.
 288. Zollner G, Halilbasic E, Wagner M, Fickert P, Silbert D, Gumhold J, Fuchsbichler A, et al. Cholic acid (CA) and ursodeoxycholic acid (UDCA) exert different effects on hepatic and renal expression of Mrp4 and Mrp6, two novel ABC transporters. *Gastroenterology* 2004; 126[4, Suppl. 2], A-16.
 289. Marschall HU, Wagner M, Zollner G, Fickert P, Gumhold J, Silbert D, Diczfalussy U, et al. Comprehensive study of the effects of rifampicin and ursodeoxycholic acid on genes and proteins involved in regulation, transport and detoxification of biliary compounds in gallstone patients. *Gastroenterology* 2004; 126[4, Suppl 2], A746.
 290. Bodin K, Bretillon L, Aden Y, Bertilsson L, Broome U, Einarsson C, Diczfalussy U. Antiepileptic drugs increase plasma levels of 4beta-hydroxycholesterol in humans: evidence for involvement of cytochrome p450 3A4. *J Biol Chem* 2001; 276(42): 38685-9.
 291. Ellis E, Axelson M, Abrahamsson A, Eggertsen G, Thorne A, Nowak G, Ericzon BG, et al. Feedback regulation of bile acid synthesis in primary human hepatocytes: evidence that CDCA is the strongest inhibitor. *Hepatology* 2003; 38(4): 930-8.
 292. Marschall HU, Wagner M, Zollner G, Fickert P, Gumhold J, Silbert D, Fuchsbichler A, et al. FXR-/- mice defend against bile acid injury by enhanced phase I detoxification. *Gastroenterology* 2004; 126[4, Suppl. 2], A-674.
 293. Kuipers F, Claudel T, Sturm E, Staels B. The Farnesoid X Receptor (FXR) as Modulator of Bile Acid Metabolism. *Rev Endocr Metab Disord* 2004; 5(4): 319-26.
 294. Pellicciari R, Fiorucci S, Camaioni E, Clerici C, Costantino G, Maloney PR, Morelli A, et al. 6alpha-ethyl-chenodeoxycholic acid (6-ECDCA), a potent and selective FXR agonist endowed with anticholestatic activity. *J Med Chem* 2002; 45(17): 3569-72.
 295. Fiorucci S, Clerici C, Antonelli E, Orlandi S, Goodwin B, Sadeghpour B, Sabatino G, et al. Protective Effects of 6-Ethyl Chenodeoxycholic Acid, A Farnesoid X receptor (FXR) Ligand, In Estrogen Induced Cholestasis. *J Pharmacol Exp Ther* 2005.
 296. Fiorucci S, Antonelli E, Rizzo G, Renga B, Mencarelli A, Ricardi L, Orlandi S, et al. The nuclear receptor SHP mediates inhibition of hepatic stellate cells by FXR and protects against liver fibrosis. *Gastroenterology* 2004; in press.
 297. Bachs L, Pares A, Elena M, Piera C, Rodes J. Comparison of rifampicin with phenobarbitone for treatment of pruritus in biliary cirrhosis. *Lancet* 1989; 1(8638): 574-6.
 298. Bachs L, Pares A, Elena M, Piera C, Rodes J. Effects of long-term rifampicin administration in primary biliary cirrhosis. *Gastroenterology* 1992; 102(6): 2077-80.
 299. Prince MI, Burt AD, Jones DE. Hepatitis and liver dysfunction with rifampicin therapy for pruritus in primary biliary cirrhosis. *Gut* 2002; 50(3): 436-9.
 300. Becker M, von BK, Rothauwe HW, Leiss O. Effects of phenobarbital on biliary lipid metabolism in children with chronic intrahepatic cholestasis. *Eur J Pediatr* 1984; 143(1): 41-4.
 301. Bloomer JR, Boyer JL. Phenobarbital effects in cholestatic liver diseases. *Ann Intern Med* 1975; 82(3): 310-7.
 302. Stiehl A, Thaler MM, Admirand WH. Effects of phenobarbital on bile salt metabolism in cholestasis due to intrahepatic bile duct hypoplasia. *Pediatrics* 1973; 51(6): 992-7.
 303. Stiehl A, Thaler MM, Admirand WH. The effects of phenobarbital on bile salts and bilirubin in patients with intrahepatic and extrahepatic cholestasis. *N Engl J Med* 1972; 286(16): 858-61.
 304. Sharp HL, Mirkin BL. Effect of phenobarbital on hyperbilirubinaemia, bile acid metabolism, and microsomal enzyme activity in chronic intrahepatic cholestasis of childhood. *J Pediatr* 1972; 81(1): 116-26.
 305. Marek CJ, Tucker SJ, Konstantinou DK, Elrick LJ, Haefner D, Sigalas C, Murray GI, et al. Pregnenolone 16alpha carbonitrile inhibits rodent liver fibrogenesis via PXR-dependent and PXR-independent mechanisms. *Biochem J* 2004.
 306. Guo GL, Moffit J, Nicol CJ, Ward JM, Aleksunes L, Slitt A, Klierwer SA, et al. Enhanced Acetaminophen Toxicity by Activation of the Pregnane X Receptor. *Toxicol Sci* 2004.
 307. Maglich JM, Watson J, McMillen PJ, Goodwin B, Willson TM, Moore JT. The nuclear receptor CAR is a regulator of thyroid hormone metabolism during caloric restriction. *J Biol Chem* 2004; 279(19): 19832-8.
 308. Yamamoto Y, Moore R, Goldsworthy TL, Negishi M, Maronpot RR. The orphan nuclear receptor constitutive active/androstane receptor is essential for liver tumor promotion by phenobarbital in mice. *Cancer Res* 2004; 64(20): 7197-200.
 309. Masuyama H, Hiramatsu Y, Kodama J, Kudo T. Expression and potential roles of pregnane X receptor in endometrial cancer. *J Clin Endocrinol Metab* 2003; 88(9): 4446-54.

310. Ritzel U, Leonhardt U, Nather M, Schafer G, Armstrong VW, Ramadori G. Simvastatin in primary biliary cirrhosis: effects on serum lipids and distinct disease markers. *J Hepatol* 2002; 36(4): 454-8.
311. Ohmoto K, Mitsui Y, Yamamoto S. Effect of bezafibrate in primary biliary cirrhosis: a pilot study. *Liver* 2001; 21(3): 223-4.
312. Kanda T, Yokosuka O, Imazeki F, Saisho H. Bezafibrate treatment: a new medical approach for PBC patients? *J Gastroenterol* 2003; 38(6): 573-8.
313. Nakai S, Masaki T, Kurokohchi K, Deguchi A, Nishioka M. Combination therapy of bezafibrate and ursodeoxycholic acid in primary biliary cirrhosis: a preliminary study. *Am J Gastroenterol* 2000; 95(1): 326-7.
314. Miranda S, Vollrath V, Wielandt AM, Loyola G, Bronfman M, Chianale J. Overexpression of mdr2 gene by peroxisome proliferators in the mouse liver. *J Hepatol* 1997; 26(6): 1331-9.
315. Hooiveld GJ, Vos TA, Scheffer GL, Van GH, Koning H, Bloks V, Loot AE, et al. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins) induce hepatic expression of the phospholipid translocase mdr2 in rats. *Gastroenterology* 1999; 117(3): 678-87.
316. Carrella M, Feldman D, Cogoi S, Csillaghy A, Weinhold PA. Enhancement of mdr2 gene transcription mediates the biliary transfer of phosphatidylcholine supplied by an increased biosynthesis in the pravastatin-treated rat. *Hepatology* 1999; 29(6): 1825-32.
317. Kok T, Bloks VW, Wolters H, Havinga R, Jansen PL, Staels B, Kuipers F. Peroxisome proliferator-activated receptor alpha (PPARalpha)-mediated regulation of multidrug resistance 2 (Mdr2) expression and function in mice. *Biochem J* 2003; 369(Pt 3): 539-47.
318. Post SM, Groenendijk M, Solaas K, Rensen PC, Princen HM. Cholesterol 7alpha-hydroxylase deficiency in mice on an APOE*3-Leiden background impairs very-low-density lipoprotein production. *Arterioscler Thromb Vasc Biol* 2004; 24(4): 768-74.
319. Weitz-Schmidt G. Statins as anti-inflammatory agents. *Trends Pharmacol Sci* 2002; 23(10): 482-6.
320. Leuschner M, Guldutuna S, You T, Hubner K, Bhatti S, Leuschner U. Ursodeoxycholic acid and prednisolone versus ursodeoxycholic acid and placebo in the treatment of early stages of primary biliary cirrhosis. *J Hepatol* 1996; 25(1): 49-57.
321. Leuschner M, Maier KP, Schlichting J, Strahl S, Herrmann G, Dahm HH, Ackermann H, et al. Oral budesonide and ursodeoxycholic acid for treatment of primary biliary cirrhosis: results of a prospective double-blind trial. *Gastroenterology* 1999; 117(4): 918-25.
322. Mitchison HC, Palmer JM, Bassendine MF, Watson AJ, Record CO, James OF. A controlled trial of prednisolone treatment in primary biliary cirrhosis. Three-year results. *J Hepatol* 1992; 15(3): 336-44.
323. Warskulat U, Kubitz R, Wettstein M, Stieger B, Meier PJ, Haussinger D. Regulation of bile salt export pump mRNA levels by dexamethasone and osmolarity in cultured rat hepatocytes. *Biol Chem* 1999; 380(11): 1273-9.
324. Jung D, Fantin AC, Scheurer U, Fried M, Kullak-Ublick GA. Human ileal bile acid transporter gene ASBT (SLC10A2) is transactivated by the glucocorticoid receptor. *Gut* 2004; 53(1): 78-84.