The Role of FXR in Disorders of Bile Acid Homeostasis

As ligands for the nuclear receptor FXR, bile acids regulate their own synthesis, transport, and conjugation, thus protecting against bile acid toxicity. Recently, the role of genetic variants in FXR itself, FXR target genes, and regulators of FXR in the pathophysiology of the liver and intestine has become increasingly evident.

Members of the nuclear receptor superfamily of transcription factors are the chief regulators of a wide variety of important metabolic pathways (28). Their ability to sense and respond to changes in intracellular metabolic environment is largely due to the fact that the transcriptional potential of most nuclear receptors is crucially dependent on small lipophilic ligands, such as bile acids, fatty acids, lipophilic vitamins, and steroidal hormones. Binding of agonistic or antagonistic ligands leads to allosteric changes in the ligand-binding domains of nuclear receptors, thus resulting in alterations in the interactions of nuclear receptors with their coactivators and corepressors, and consequently affecting transcriptional rates of target genes (88). Most nuclear receptors bind to their DNA response elements as either hetero- or homodimers, and their consensual DNA-binding motifs typically contain two hexameric half sites. These hexameric motifs, the consensus sequence for which is AGGTCA, can be arranged as direct (DR), inverted (IR), or everted (ER) repeats, separated by a variable and receptor-specific number of base pairs.

The suggested role of altered nuclear receptor activity in several metabolic diseases, together with the potential of modulating their activity with specific ligands, has made them attractive targets for pharmacological intervention in these diseases (29). Given that most nuclear receptors are involved in regulating a variety of metabolic processes, and accordingly have multiple target genes, the major challenge will be to achieve functional selectivity by therapeutic ligands, only affecting a desired subset of downstream target genes in specific tissue targets.

The Nuclear Receptor for Bile Acids, FXR

The farnesoid X receptor (FXR; also known as the bile acid receptor or BAR; gene symbol NR1H4) is a member of the nuclear receptor family of transcription factors. FXR functions as the chief sensor of intracellular levels of bile acids (the end products of cholesterol catabolism) and the main executor of bile acid-induced transcriptional programmes. Bile acids directly interact with the ligand-binding domain of FXR and enhance or antagonize the transactivation function of FXR. In accordance with its function as the bile acid receptor, FXR is most abundantly expressed in the tissues commonly exposed to bile acids in normal physiology: liver, intestine, and kidneys (25). Along the intestinal tract, higher FXR levels can be found in the ileal epithelium, the main site of intestinal bile acid absorption, than in the epithelium of proximal small intestine or the colon (39). The preferred DNA-binding sequence for FXR within its target promoters is typically a variant of the so-called “inverted repeat-1” motif (IR-1; inverted hexameric AGGTCA-like repeat separated by one base pair) (57), to which FXR binds as a heterodimer with the nuclear retinoid X receptor (RXR). Although in lower mammals there are two Fox genes, Nr1h4 (encoding Fxr) and Nr1h5 (encoding Par1p), the latter gene product employing lanosterols as its ligands, in humans and other primates the homolog of Par1p is a pseudogene (76). Thus, in this review, focusing on the properties and function of this nuclear receptor in humans, we use the phrase FXR/Fxr to refer to FXR/Fxr.

Four isoforms of FXR, termed FXRα1–4, can be translated from the single NR1H4 gene in humans, generated by both alternative promoter usage and by alternative splicing (37, 109). FXRα1 and FXRα2, the most abundant FXR isoforms in the human liver, differ from FXRα3 and FXRα4 at the NH2 terminus, since the mRNAs for these two isoform pairs are transcribed from separate promoters. In addition, the isoforms FXRα1 and FXRα2 contain an additional stretch of four amino acids, MYTG, due to a differential splicing event at the end of exon 5. All four FXR isoforms harbor identical ligand-binding domains but may exhibit differential DNA-binding and transactivation properties (62, 66, 79). In the context of most FXR target genes this remains to be comprehensively studied.

FXR functions typically as an agonist-dependent transcriptional activator of its direct target genes. Numerous transcriptional coactivators recruited to agonist-bound FXR, mediating its transcriptional function, have been proposed (reviewed in Ref. 26). FXR can also negatively regulate transcription of specific target genes in an agonist-dependent manner. Although there is evidence from the studies on the human apoA1 promoter that FXR binding can activate gene expression via typical nuclear receptor ligands (88). The SHP-1 protein, mediated through its SH2 domain and an atypical nuclear receptor ligand, can interact with the nuclear receptor family and regulate gene expression (11, 13). SHP-1 binds to FXR, which may suppress a transcriptional activator or promote transcriptional repression (50).

The farnesoid X receptor family is highly conserved across mammalian species (21). In humans, there are two FXR genes, NR1H4 and NR1H5. The latter gene product employs lanosterols as its ligands, while NR1H4 is the principal FXR gene in mammals.

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human apolipoprotein A-I and apolipoprotein C-III promoters that this may be achieved through direct binding by FXR to negative biliary acid response elements (11, 13), more often FXR downregulates target genes via an indirect mechanism involving another nuclear receptor, small heterodimer partner (SHP) (88). The SHP (NR0B2) gene is directly transactivated by FXR in response to FXR ligands and encodes an atypical nuclear receptor, lacking the DNA-binding domain. However, SHP does contain the dimerization domain and a putative ligand-binding domain. SHP can effectively recruit a transcriptional repressor complex to the target promoters (50, 61), or both. No endogenous or exogenous ligands for SHP have been confirmed to exist. However, a synthetic retinoid termed CD457/AHPN was recently shown to both directly interact with SHP and enhance the recruitment of a corepressor complex (21), although it is not yet clear whether the interaction with this compound is in fact mediated by the putative ligand-binding pocket of SHP.

FXR Ligands: Bile Acids and Beyond

Nuclear receptors, for which no ligands have yet been identified, have been termed “orphan nuclear receptors.” The first attempt to rescue FXR from the orphanage (i.e., to find its ligand) was made in 1995, when farnesol metabolites were suggested as candidates to be such FXR ligands; however, these compounds only activate FXR at concentrations clearly exceeding physiological conditions and do not appear to act as bona fide direct ligands for FXR (25, 107). FXR was finally adopted in 1999, when three groups reported that bile acids belong to the isoforms NR1H4 and NR1H5 (76), to which the term “inverted atypical nuclear receptor” was applied (57), to which other endogenous ligands have been referred (125, 127). However, SHP does contain the dimerization domain and a putative ligand-binding domain. However, SHP does contain the dimerization domain and a putative ligand-binding domain. SHP can effectively recruit a transcriptional repressor complex to the target promoters (50, 61), or both. No endogenous or exogenous ligands for SHP have been confirmed to exist. However, a synthetic retinoid termed CD457/AHPN was recently shown to both directly interact with SHP and enhance the recruitment of a corepressor complex (21), although it is not yet clear whether the interaction with this compound is in fact mediated by the putative ligand-binding pocket of SHP.

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The preferred ligands are primary bile acids in mammals there (25, 57), and SHP acts employing other endogenous (126). Thus, the isoform-specific ligands and function reflect sequence divergence of the phrase

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Therapeutic usage of choleretic FXR ligands that leads to increased bile flow via the regulatory mechanisms described below may not be advantageous in cholestatic diseases that frequently have an obstructive component: in a mouse study, increased bile flow and biliary pressure in fact aggravated biliary infarcts in mice suffering from obstructive cholestasis upon bile duct ligation (23).

**FXR Controls and Fine-Tunes Bile Acid Homeostasis**

Bile acids are the end products of hepatic cholesterol catabolism, thus providing a major pathway of excess cholesterol elimination from the body. Approximately 500 mg of bile acids are synthesized every day in the adult human liver. Enterohepatic cycling of bile acids, mediated by plasma membrane transporters expressed in hepatocytes and enterocytes in a polarized manner, is highly efficient in healthy individuals, with approximately only 5% escaping ileal reabsorption and being lost into feces. Functioning as physiological regulators, bile acids promote absorption of lipophilic nutrients and vitamins in the intestine. Furthermore, bile acids contribute to the solubilization of cholesterol in bile, thus protecting against precipitation of cholesterol crystals and preventing the formation of cholesterol gallstones. Despite these crucially important roles in normal physiology, elevated levels of bile acids, such as observed in cholestatic disease, can be cytotoxic due to their detergent properties. To avoid cellular damage, bile acids function as homeostatic regulators and signaling molecules to adjust their own intracellular levels. Bile acids exert their regulatory effects chiefly by acting as FXR ligands, although they do also elicit other signaling pathways, such as those involving a G-protein-coupled receptor TRGR (48) and the c-Jun NH2-terminal kinase (JNK) (30), which are likely to contribute to the protection against bile acid-induced damage. It is interesting to note that different bile acids have differing affinities to FXR and TGR5 (85), providing one mechanism for the divergent downstream signaling events that they elicit.

In the liver and intestine, the transcriptional events exerted by bile acid-activated FXR lead to increased cellular bile acid efflux and detoxification and decreased bile acid uptake and synthesis, as described below. **FXR induces bile acid efflux and detoxification machinery**

The ABCB11 gene encoding BSEP, the chief liver canicular bile salt export pump of the ATP-binding cassette (ABC) transporter family (90), is a target for direct transactivation by FXR (2, 81, 87) (FIGURE 1; Table 1). Thus, in conditions of increased bile acid load in hepatocytes, bile acids enhance their own efflux into bile by activating FXR and consequently increasing BSEP expression. Although BSEP is responsible for the efflux of monovalent bile acids from hepatocytes into bile, the multidrug resistance-associated protein 2 (MRP2, ABCC2) contributes to the overall canalicular bile acid efflux by exporting divalent and sulphated or glucuronidated bile acids into bile (reviewed in Ref. 53). Both the human and rodent ABCC2/Abcc2 promoters can be activated by FXR in the presence of bile acids (47). Yet another ABC transporter gene that is transactivated by FXR is ABCB4, encoding MDR3 (35), which is thought to be a floppase for phospholipids within the canalicular membrane of hepatocytes. Via induction of ABCB4 expression, FXR enhances the function of MDR3 in counteracting the toxicity of biliary bile acids by promoting formation of mixed micelles that contain cholesterol, bile acids, and phospholipids in bile canaliculi.

The SLC20A1 gene encoding a member of the organic anion transporting polypeptide (OATP) family, OATP1B1, is directly transactivated by FXR in a ligand-dependent manner through an IR-1 element (45). OATP1B1 is an uptake system at the sinusoidal membrane of hepatocytes for numerous drugs and peptides, such as digoxin and cholecystokinin (40, 55). OATP1B1 is also transport bile acids in a sodium-independent manner (31), although the extent of its contribution to overall bile acid transport into hepatocytes has not been established. As discussed below, the activity of another liver-specific basolateral uptake system with an overlapping substrate specificity, OATP1B3, is suppressed by FXR-dependent pathways in cholestatic, bile acid-enriched environment (44). Induction of OATP1B3 expression by FXR may serve to maintain sufficient hepatic extraction of organic solutes and xenobiotics in conditions that lead to decreased expression of other basolateral drug uptake transporters, such as OATP1B1 (56).

In an analogous manner to the ABCB11 gene, FXR induces the expression of the two genes encoding the essential heterodimeric bile acid efflux system at the basolateral membrane of ileocytes, OSTA/OSTj1 (Refs. 58, 59, 82; Table 2). The OSTA/OSTj1 heterodimer has also been suggested to facilitate biliary excretion of human bile acids from bile ductular epithelial cells and thus be involved in the regulation of FXR-mediated bile acid detoxification (19). Furthermore, OSTA/OSTj1 expression is increased in primary biliary cirrhosis (62) and increased in bile duct ligation-induced bile acid-dependent cholestasis, providing one mechanism for the divergent expression of OSTA/OSTj1 during cholestasis in different species.  

**Table 1. Hepatic genes discussed in the review, the FXR-dependence of their expression, and their functions**

<table>
<thead>
<tr>
<th>Gene</th>
<th>FXR Target</th>
<th>Known or Proposed Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSEP</td>
<td>+</td>
<td>Major canicular bile acid efflux system</td>
</tr>
<tr>
<td>NTCP</td>
<td></td>
<td>Major sinusoidal bile acid uptake system</td>
</tr>
<tr>
<td>MDR3</td>
<td>+</td>
<td>Canicular phospholipid efflux system</td>
</tr>
<tr>
<td>MRP2</td>
<td>+</td>
<td>Canicular drug and bile acid efflux system</td>
</tr>
<tr>
<td>OATP1B1</td>
<td>+</td>
<td>Sinusoidal drug and bile acid uptake system</td>
</tr>
<tr>
<td>OATP1B3</td>
<td>+</td>
<td>Sinusoidal drug and peptide uptake system</td>
</tr>
<tr>
<td>OSTa/OSTj1</td>
<td>+</td>
<td>Sinusoidal alternative bile acid efflux system</td>
</tr>
<tr>
<td>CYPIA1</td>
<td>+</td>
<td>Bile acid synthesis via cholesterol catabolism</td>
</tr>
<tr>
<td>CYPB1</td>
<td>+</td>
<td>Bile acid synthesis via cholesterol catabolism</td>
</tr>
<tr>
<td>CYPJ2A1</td>
<td>+</td>
<td>Bile acid synthesis via cholesterol catabolism</td>
</tr>
<tr>
<td>UGT2B4</td>
<td>+</td>
<td>Glucuronidation of bile acids</td>
</tr>
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</table>

For details and references to literature, please see the main text. +, induced by FXR; −, suppressed by FXR.
The chief liver ATP-binding cassette (ABC) transporter involved in the uptake and efflux of bile acids is the bile salt export pump (BSEP) (Ref. 19). BSEP is responsible for the export of bile acids associated with the excretion of cholesterol; it is expressed in intestinal and hepatic cell lines, OSTa and OSTβ, gene expression, and is induced upon bile acid treatment of biliary samples derived from the human ileal tissue (Ref. 58). Physiological support for these in vitro and ex vivo studies is provided by the finding that bile acid-induced expression of the human OSTa/OSTβ gene expression in the ileal tissue of a study group consisting of female nonobese gallstone disease patients (Ref. 83). In addition to the bile acid efflux systems, FXR also transactivates genes encoding enzymes that can metabolize and thus detoxify bile acids. One such FXR target is the gene encoding the human uridine 5'-diphosphate-glucuronosyltransferase 2B4 (UGT2B4) enzyme, which converts hydrophobic bile acids to more hydrophilic glucuronide derivatives via an increase in the negative charge of a bile acid molecule (Ref. 4). The UGT2B4 promoter is activated by FXR binding DNA as a monomer, without its heterodimerization partner RXR.（Ref. 19）

**FIGURE 1. Membrane transporters and other proteins expressed in the liver or intestine, the expression of which is regulated by FXR.** The location of the membrane proteins at the correct membrane domain is shown in the divergent species via distinct mechanisms.

**REVIEWS**

The Na+-taurocholate co-transporting polypeptide (NTCP) is the predominant transporter responsible for bile acid uptake from portal blood across the basolateral membrane of hepatocytes. In rodent models of cholestasis, expression of the Ntcp mRNA and protein is notably decreased (Ref. 22, 27, 112). Thus, in addition to enhancing bile acid efflux systems and bile acid synthesis, FXR negatively regulates bile acid uptake systems and bile acid synthesis.

**FIGURE 1.** Membrane transporters and other proteins expressed in the liver or intestine, the expression of which is regulated by FXR. The location of the membrane proteins at the correct membrane domain is shown in the divergent species via distinct mechanisms.
proposed pathway emphasizes the coordinated regula-
tion of the bile salt uptake systems in both human liver and intestine.
In addition to downregulating the expression of bile acid uptake transporters in the liver and the intestine, FXR also represses transcription of three genes coding for bile acid synthesizing enzymes, namely cholest-
terol-7α-hydroxylase (CYP7A1), sterol-12α-hydroxy-
lase (CYP8B1), and sterol-27-hydroxylase (CYP27A1), in a ligand-dependent manner (reviewed in Ref. 20).
Thus elevated levels of bile acids can suppress their own de novo production through a negative feedback loop. All three CYP promoters contain a negative bile acid response element, which is targeted by the FXR-
induced repressor SHP. In the CYP7A1 and CYP8B1 promoters, the targeted DNA-binding transactivators are the monomeric nuclear receptor liver receptor homolog-1 (L-RH-1) and homodimeric HNF-4α, which have overlapping DNA-binding motifs on both pro-
motors. In the case of the CYP27A1 gene, the negative bile acid response element contains a DNA-binding site only for HNF-4α but not for L-RH-1.
Although SHP-deficient mice exhibit impaired nega-
tive feedback regulation of bile acid synthesis, this is not completely abolished, implying that additional or parallel SHP-independent repression pathways may lead to reduced bile acid synthesis (51, 100). One such alternative pathway involving signaling between the liver and the intestine has been proposed: in response to bile acids, FXR directly activates the gene encoding Bile Acid-Inducible Growth Factor (FXR; –, suppressed by FXR).

FGF19/Fgf15 is released to portal blood, and upon reaching the liver it elicits a signaling cascade by bind-
ing to the fibroblast growth factor receptor-4 (FGFR4) tyrosine kinase on the surface of hepatocytes. Activated FGFR4 stimulates the intracellular JNK kinase path-
way, which eventually suppresses the CYP7A1 promot-
er in the nucleus. A complex mechanism has been proposed for this downstream effect, according to which JNK signaling induces expression of the tran-
scription factor c-Jun, which via a direct interaction with HNF-4α may block the recruitment of the tran-
scriptional coactivator PGC-1α, thus resulting in sup-
pression of CYP7A1 gene expression (63). It appears that SHP somehow contributes to the FXR-

FGF19/Fgf15 pathway of repression of bile acid synthe-
sis, since the effect seems clearly attenuated in SHp-
deficient mice (38). The FGF19/Fgf15-mediated endocytosis-type loop may explain the previous observa-
tions that intestinal administration of bile acids leads to decreased hepatic CYP7A1 expression in rats, whereas intravenous or portal administration does not (73, 78).

Genetic Variation in the FXR Gene
in Liver and Biliary Diseases

Genetic mutations and polymorphisms in several members of the nuclear receptor family, such as per-
oxisome proliferator-activated receptors-α and -γ (PPARα, PPARγ; Refs. 70, 91), vitamin D receptor (VDR; Ref. 95), and hepatocyte nuclear factor-4α (HNF-4α; Ref. 105) have been associated with specific metabolic disorders. There are currently approximately 400 genetic single nucleotide polymorphisms (SNPs) or mutations within the NR1H4 gene encoding FXR submitted to the NCBI (http://www.ncbi.nlm.nih.gov/snp) and HapMap (http://www.hapmap.org) SNP databases, most of them located in the intronic regions or in regions flanking the FXR coding sequence. Only recently have reports been published on associations between genetic variants in the NR1H4 gene and human pathobiology, and these are summarized below.

Intrahepatic cholestasis of pregnancy

In the study by van Mil et al. (97), both the coding regions and exon/intron boundaries of the NR1H4 gene were studied in 92 British women of varied eth-
nic origins, who were suffering from intrahepatic cholestasis of pregnancy (ICP). ICP is a reversible form of a cholestatic disease, which is frequently associated with adverse pregnancy outcomes, such as premature birth, fetal distress, and intra-uterine death. Four heter-
ozygous NR1H4 variants were identified: –1G>T, 1A>G (M1V), 238T>C (W80R), and 518T>C (M173T). The first two FXR variants, –1G>T and 1A>G (M1V), were shown to lead to reduced FXR protein expression and decreased level of transcriptional activation of a FXR-
dependent promoter construct in transfected human embryonic kidney HEK293T cells compared with the wild-type FXR. The 518T>C (M173T) variant, harboring an amino acid substitution within the zinc finger DNA-binding domain of FXR, also led to a reduction in the transcriptional activity of FXR in cell-based assays, even if the degree of protein expression, DNA-binding, and heterodimerization with RXR remained apparently unaffected by the change of residues. Another group published simultaneously an independent study on FXR polymorphisms in populations of European, African, Chinese, and Hispanic descent (68). One of the four FXR variants identified in this latter study also contained the –1G>T substitution in the base position adjacent to the translational initiation site. Although in this study the SNPs were associated with an increased prevalence of gallstones and a reduced liver function, they were not evaluated con-
eductively and in a population to which the previ-
ous studies were limited, the patients in the current study were exclusively from British ICP pa-
tients (97), a case-control study design was employed, and the control group was matched on the basis of age, parity, and mode of delivery. Out of the two patients carrying the FRX-1A>G (M1V) and 518T>C (M173T) variants, both showed an increased frequency of high sensitivity C reactive protein (hs-CRP) over the duration of pregnancy, while the other groups did not show any significant difference. The authors concluded that the FRX-1A>G (M1V) and 518T>C (M173T) variants may be useful for the identification of women at risk for developing ICP.
Cholesterol gallstone disease

HapMap (http://www.hapmap.org) provides resources for genome-wide studies of genetic variation in human populations, and the Human Genome Diversity Project has provided a sampling of genetic diversity from 104 human populations. The Human Protein Atlas (http://www.proteinatlas.org) and the In Silico Analysis of Human Gene Expression (http://www.in-silico.org) provide resources for protein expression, function, and regulation, and the Nucleic Acid Data Bank (http://www.ncbi.nlm.nih.gov/snp) and HapMap (http://www.hapmap.org) provide extensive SNP data on single nucleotide polymorphisms within the NR1H4 gene.

The current study suggests that genetic variation in the FXR gene may contribute to the development of cholesterol gallstone disease.
cholesterol gallstone disease, such as supersaturation of cholesterol in bile, precipitation of cholesterol crystals in the gallbladder, and increased hydrophobicity of bile salts (32, 71). Furthermore, in a gallstone-susceptible FXR wild-type mouse strain, application of the specific FXR ligand GW4064 reduced gallstone prevalence by increasing biliary bile salt and phospholipid concentrations and restoring cholesterol solubility.

It is clear that other regulatory factors are also involved in the development of the undoubtedly multifactorial gallstone disease. In a recent report of a mouse study (7), the winged helix/forkhead transcription factor FoxO1 was shown to be a positive regulator of the familial intrahepatic cholestasis-1 (FIC1) protein, which acts as an aminophospholipid flippase, translocating phosphatidylserine from the outer leaflet of the membrane. The FIC1-FXR Connection and Intrahepatic Cholestasis.

Cholesterol cholelithiasis by increasing biliary bile salt and phospholipid concentrations and restoring cholesterol solubility.

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This study demonstrates that the functional activity of the -1G>T variant also appeared to be compromised, the level of transcription and translation efficiency of the variant was evaluated comparable to the wild-type in a cell-free assay and in transfected HeLa cells. The difference between the two studies remains unclear but may simply be due to the different experimental setups. Interestingly, the miRNA expression levels of the FXR target genes SHP and OATPIB3 are significantly reduced in the livers of the heterozygote subject carrying the -1G>T allele, whereas the FXR mRNA expression levels were similar in control subjects indicating that this polymorphism may lead to compromised function but not expression level of FXR (68).

The familial intrahepatic cholestasis-1 (FIC1) protein, encoded by the ATP8B1 gene, is expressed at the liver canalicular membrane and at the apical membrane of enterocytes, in addition to many other tissues (94). ATP8B1 acts as an aminophospholipid flippase, translocating phosphatidylserine from the outer leaflet of the lipid bilayer of the plasma membranes, thus contributing to the lipid asymmetry of the membrane. The exact significance of FIC1 in normal physiology is not known in detail, but it is believed to contribute to the detergent-resistant properties of the liver canalicular membrane.

In further support of the role for FXR in cholelithiasis, FXR-null mice exhibit the typical characteristics of cholesterol gallstone disease, such as supersaturation of cholesterol in bile, precipitation of cholesterol crystals in the gallbladder, and increased hydrophobicity of bile salts (32, 71). Furthermore, in a gallstone-susceptible FXR wild-type mouse strain, application of the specific FXR ligand GW4064 reduced gallstone prevalence by increasing biliary bile salt and phospholipid concentrations and restoring cholesterol solubility.

It is clear that other regulatory factors are also involved in the development of the undoubtedly multifactorial gallstone disease. In a recent report of a mouse study (7), the winged helix/forkhead transcription factor FoxO1 was shown to be a positive regulator of the genes encoding the heterodimeric cholesterol transporters ABCG5/ABCG8 at the canalicular membrane and at the apical membrane of enterocytes, in addition to many other tissues (94). ATP8B1 acts as an aminophospholipid flippase, translocating phosphatidylserine from the outer leaflet of the lipid bilayer of the plasma membranes, thus contributing to the lipid asymmetry of the membrane. The exact significance of FIC1 in normal physiology is not known in detail, but it is believed to contribute to the detergent-resistant properties of the liver canalicular membrane.

Also in the above-mentioned report by van Mil et al. (97), a case-control study of a British cohort (293 ICP cases, 290 controls) and a Swedish cohort (49 ICP cases, 59 controls) was performed to determine the allelic frequencies of the NR1H4 variants previously identified. Out of the two variants present in both the ICP group and the control group, -1G>T and 51H-T-C (M173T), only the latter exhibited significant associations with the ICP phenotype in the Swedish group. It is clear that in such genetic association studies larger cohorts will be needed to confirm the results and to discover further rare disease-associated variants. Furthermore, as cholesterol gallstone diseases may be complex, possibly requiring particular allelic variants in multiple susceptibility or modifier loci or certain environmental influences, further investigations into the potentially combinatorial nature of these diseases will be needed.

Consistent with FXR-stimulated transcriptional pathways being affected in ICP, genetic variants of the well-known FXR target genes ABCB4, encoding BSEP, and ABCG4, encoding MDR3, have also been associated with this pathological condition in several studies (17, 49, 69, 80, 86). Furthermore, variants of the gene itself via coactivation of the nuclear receptors PPARγ and RXR, as well as the coactivator of FXR, mediating the activation of FXR target genes (6). However, in this latter study, the allelic status of the NR1H4 gene was not determined in the patients. Although the expression of FXR itself was not altered in this group of gallstone patients, the transcriptional coactivator protein PGC-1α was significantly less expressed in the livers of patients with cholesterol cholelithiasis when compared with nongallstone patients. PGC-1α can function as a coactivator of FXR, mediating the activation of FXR target genes (46, 108), and may also enhance the expression of the NR1H4 gene itself via coactivation of the nuclear receptors PPARγ and HNF-4α (108). Reduced expression of PGC-1α in gallstone patients could thus lead to decreased expression of both FXR and its target genes BSEP and MDR3, the actions of which help to maintain cholesterol in its soluble form in the bile.

In further support of the role for FXR in cholelithiasis, FXR-null mice exhibit the typical characteristics of cholesterol gallstone disease, such as supersaturation of cholesterol in bile, precipitation of cholesterol crystals in the gallbladder, and increased hydrophobicity of bile salts (32, 71). Furthermore, in a gallstone-susceptible FXR wild-type mouse strain, application of the specific FXR ligand GW4064 reduced gallstone prevalence by increasing biliary bile salt and phospholipid concentrations and restoring cholesterol solubility.

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plasma membrane. Genetic mutations and polymor-
phisms in the ATP8B1 gene have been associated with
familial intrahepatic cholestasis, characterized by low γ-glutamyltranspeptidase plasma levels (reviewed in Refs. 41, 96). A disease entity termed progressive familial
intrahepatic cholestasis type 1, or PFIC1 (also known as “Byler’s disease”) is the severe form of PFIC-related liver disease, characterized by fat malabsorption, intense pruritus, and frequently leads to the cirrhosis of the liver. The milder form of PFIC-related disease is
named benign recurrent intrahepatic cholestasis type 1, or BRIC1. In BRIC1, discrete cholestatic episodes are separated by asymptomatic periods, and BRIC1 does not typically lead to progressive liver injury. PFIC1 and
BRIC1 are associated with distinct sets of genetic vari-
ants in the ATP8B1 gene, and it could be hypothesized that the severity of the disease may correlate with the degree of corresponding effect in PFIC1 function.

In 2004, two groups reported that, in PFIC1 patients, there is a tendency for decreased hepatic and intestinal mRNA levels of FXR and of genes transactivated by FXR (1, 10), implying that FXR may influence the expression and/or function of FXR, possibly thus contributing to the pathogenesis of the liver disease. Intriguingly, in a more recent report, it was shown that, whereas the wild-type FIC1 was capable of potent activation of the β3EP pro-
motor, the PFIC1-associated β3EP variants were inactive, and the BRIC1-associated β3EP variants activated the β3EP promoter to a moderate degree (26). The authors further hypothesized that the wild-type FIC1 protein induces nuclear localization of FXR through stimulation of a phosphorylation cascade targeting FXR and that FIC1-related disease may be caused by the compro-
mised ability of the associated β3EP variants to influence FXR localization and function.

FXR-Mediated Indirect Mechanisms Affecting Intestinal Bacterial Growth

It was previously known that bile acids can protect against bacterial overgrowth in the small intestine via their anti-microbial activity, thus helping to maintain the integrity of the intestinal epithelium and to pre-
vent systemic infections caused by bacterial transloca-
tion across the mucosal barrier (5, 65). In mice, bile
acids exert this protection against intestinal mucosal injury via FXR (39). In wild-type mice, bile duct ligation resulted in a significant increase in the number of intestinal bacteria and led to bacterial invasion of the mucosa. Administration of the synthetic and potent FXR ligand GW4064 alleviated these effects of bile duct ligation in wild-type but not FXR-deficient mice. In gene profiling studies in mice, several candidate GW4064-induced genes were identified that could potentially be involved in the intestinal mucosal defence. Perhaps one of the most notable of these is the gene encoding the inducible nitric oxide synthase (iNOS), given the antimicrobial properties of nitric oxide, as well as its role in epithelial barrier function (74, 98). Another gene identified as GW4064-inducible was the gene encoding angiotensin, which also exerts anti-bacterial effects (34). It may be that antigenic lig-
ands for FXR could be therapeutically useful in patients with reduced bile flow and consequently ele-
Vation of bacterial growth and invasion across the intes-
tinal mucosa.

Outlook

At present, the physiological significance of FXR function and expression is largely supported by observations in FXR-deficient mice. As crucial as such rodent whole-organism studies are, their results should be interpreted with a certain degree of caution: even slight differences in the complex promoter regions of FXR target genes between species may result in differential transcriptional conse-
quences of FXR activation, and thus differential physiologic downstream effects. Furthermore, since FXR-null mice have been devoid of any FXR function throughout their embryonic development and adult life, compensatory mechanisms may have developed. These models may not accurately reflect the situation in human pathologies, where genetic variants of FXR may have altered, but by no means are absent in, function. Increased knowledge of the consequences of genetic variation in the human FXR gene itself, FXR target genes, or potential upstream regulators of FXR such as FIC1, and of association between these genetic events and human pathogen-
esis should assist us in our understanding of the pathobiology of these diseases and point to new therapeutic targets.

In addition to the bile acid homeostasis in the liver and intestine discussed here, FXR has been identified as a key player in several other metabolic processes, such as glucose and lipid metabolism (12), as well as carcinogenesis (52, 106) and liver cell proliferation (36). Although this has raised much excitement about the potential of modulating FXR activity in pathogenic processes beyond those involving disturbed bile acid homeostasis, this has also highlighted the potential difficulties in achieving tissue, process, and even gene specificity in therapeutic targeting of FXR. Since the range of FXR ligands, endogenous and synthetic, is rapidly increasing, the likelihood of discovering FXR agonists and antagonists that may function in a more specific manner may be increased, and modeling of novel synthetic FXR ligands displaying such specificity may be facilitated.

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the function of FXR is crucially supported by its nuclear bile acid receptor activity, which is essential in the development of any FXR-dependent bile acid signaling. As crucial as FXR is in the liver, it is only recently that this nuclear receptor family has been identified as being involved in the regulation of cholesterol gallstones. As such, this review aims to highlight the potential of FXR and its role in the pathogenesis of cholesterol gallstones.

References


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