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 transactivation potential of most nuclear receptors is crucially dependent on small lipophilic ligands, such as bile acids, fatty acids, lipophilic vitamins, and steroid 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 (89). 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 reverted (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 specific number of base pairs.

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 mammalian tissues there are two FXR genes, Nr1h4 (encoding Fxr1) and Nr1h5 (encoding Par1), the latter gene product employing lanosterol as its ligands, in humans and other primates the homolog of Par1 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 FXR1/Fxr1.

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 FXRα3 and FXRα4 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 different coactivator recruitment, DNA-binding, or RXR heterodimerization properties. In the context of at least a subset of target genes, the FXR isoforms appear to exhibit differential DNA-binding and transactivation properties (4, 109), although in the context of most FXR target genes this remains to be comprehensively studied.

FXR functions typically as an agonist-dependent transcriptional transactivator of its direct target genes. Numerous transcriptional coactivators recruited to agonist-bound FXR, mediating its transcriptional function, have been proposed (reviewed in Ref. 20). 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 apolipoprotein B promoter that FXR binds to DNA via specific motifs (88). The SHP domain of FXR interacts with the SH2 domain of the adaptor protein SHIP, as well as with the SH3 domain of receptors belonging to the Src family (20). SHP may mediate the transcriptional transactivation properties of FXR (60, 62, 66, 79). FXR appears to be specifically expressed in cells of the primary bile acid biosynthetic pathway, chenodeoxycholic acid (78) and the related potent FXR agonist farnesol (62, 66, 79). Other commonly used therapeutic agents with FXR activity such as chenodeoxycholic acid (59) and the potent FXR agonist farnesol (62, 66, 79) may also act on FXR to activate FXR-dependent transcription.

Since the identification of several other nuclear receptors that recognize bile acids directly and FXR ligands include hydroxycholesterol and other bile acids as well as such an agonistic ligand as farnesol.
The only FXR isoforms expressed in humans, FXRα-1 and FXRα-2, the former being also present in other primates. FXRα-1 is the predominant isoform in the liver, while FXRα-2 is detected in the renal cortex and to a lesser extent in the small intestine (57). In contrast, the liver-specific FXR isoform is found in several other species, including rodents, to a different extent (24). When evaluating the therapeutic efficiency of synthetic FXR agonists and antagonists has developed over the last few years. In rodent models, some of these have already shown promising hepatoprotective and antithrombotic properties (15, 24). When designing FXR agonists and antagonists, the need to be selective for the FXR receptor is crucial, as FXR agonists may have side effects on other nuclear receptors involved in cholesterol homeostasis. Another strategy to overcome the potential for off-target effects is to use FXR antagonists, which may selectively block FXR activation in certain tissues or cells (41).

In addition to naturally occurring FXR ligands, several other substances have been identified that may function as FXR agonists or antagonists. For example, the bile acid derivative 6-ethyl chenodeoxycholic acid (6ECDCA) protects against liver fibrosis (48). In another study, the synthetic FXR agonist CD437/AHPN was shown to be more effective than UDCA in reducing liver fibrosis in an experimental liver fibrosis model (21). These findings suggest that the development of FXR agonists and antagonists may provide new treatment options for liver diseases, including chronic liver disease and non-alcoholic fatty liver disease (NAFLD).

In summary, FXR is a key player in the regulation of bile acid and cholesterol metabolism. Its activation by endogenous ligands leads to the repression of genes involved in cholesterol synthesis and the induction of genes involved in bile acid and cholesterol transport. The identification of synthetic FXR agonists and antagonists is an active area of research, with potential applications in the treatment of liver diseases and other metabolic disorders.
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 bile 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. Enterohydroplastic 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 detergents, 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 activating the retinoid X receptor (RXR) heterodimer. In addition, bile acids affect other signaling pathways, such as inducing oval cell proliferation, influencing bile acid synthesis and export, and decreasing expression of other basolateral drug uptake transporters, such as OATP1B1 (56).

**Table 1. Hepatic genes discussed in the review, the FXR-dependence of UGT2B4 CYP27A1 CYP8B1 CYP7A1 OST MRP2 MDR3 NTCP BSEP**

<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 canalicular 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>Canalicular phospholipid efflux system</td>
</tr>
<tr>
<td>MRP2</td>
<td>+</td>
<td>Canalicular 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>OSTα/OSTβ</td>
<td>+</td>
<td>Sinusoidal alternative bile acid efflux system</td>
</tr>
<tr>
<td>CYP7A1</td>
<td>-</td>
<td>Bile acid synthesis via cholesterol catabolism</td>
</tr>
<tr>
<td>CYP8B1</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>
</tbody>
</table>

For details and references to literature, please see the main text. +, induced by FXR; -, suppressed by FXR.

**FXR induces bile acid efflux and detoxification machinery**

The ABCB11 gene encoding BSEP, the chief liver canalicular 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 by activating FXR and consequently increasing BSEP expression. Although BSEP is responsible for the efflux of monovalent bile acids from hepatocytes into bile, the multifrug resistance-associated protein 2 (MRP2, ABCG2) 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 ABCG2/Abcg2 promoters can be activated by FXR in the presence of bile acids (47). Yet another ABC transport gene that is transactivated by FXR is ABCB4, encoding MDR3, which is thought to be a flippase for phospholipids within the canalicular membrane of hepatocytes. Via induction of ABCB4 expression, FXR enhances the function of MDR3 in transporting toxic bile acids by promoting formation of mixed micelles that contain cholesterol, bile acids, and phospholipids in bile canaliculi.

The SLC22A3 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 (43). OATP1B1 is an uptake system at the sinusoidal membrane of hepatocytes for numerous drugs and peptide hormones, such as digoxin and cholecystokinin (40, 55). OATP1B1 may 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 cholangiocytes, OSTα/OSTβ (Refs. 58, 59, 82; Table 2). The OSTα/OSTβ heterodimer has also been suggested to play a role in the enterohepatic circulation of bile acid. This is achieved by FXR induction of the other bile acid export gene, ABCB11, as well as by increasing the expression of the basolateral bile acid transporters OSTα/OSTβ, which can also transport bile acids in a sodium-independent manner (31).

In addition to bile acid transporters, OATP1B3 also transports other lipophilic solutes and xenobiotics in conditions that lead to increased bile acid concentrations. It is notable decreased expression of OATP1B3 is present in various cholestatic diseases with a common bile duct obstruction (4). Thus, induction of OATP1B3 by FXR is another example of a responsive bile acid transport system that is increased in experimental bile duct obstruction. It is notable that while bile acid concentrations are increased, bile acid concentrations are decreased in conditions of bile acid deficiency due to the treatment of cholestatic diseases with cholestyramine or the bile acid sequestrant chenodeoxycholic acid (112). Thus, induction of OATP1B3 by FXR is another example of a responsive bile acid transport system that is decreased in conditions of low bile acid concentrations. It is notable that bile acid concentrations are decreased in conditions of bile acid deficiency due to the treatment of cholestatic diseases with cholestyramine or the bile acid sequestrant chenodeoxycholic acid (112). Thus, induction of OATP1B3 by FXR is another example of a responsive bile acid transport system that is decreased in conditions of low bile acid concentrations. It is notable that bile acid concentrations are decreased in conditions of bile acid deficiency due to the treatment of cholestatic diseases with cholestyramine or the bile acid sequestrant chenodeoxycholic acid (112). Thus, induction of OATP1B3 by FXR is another example of a responsive bile acid transport system that is decreased in conditions of low bile acid concentrations. It is notable that bile acid concentrations are decreased in conditions of bile acid deficiency due to the treatment of cholestatic diseases with cholestyramine or the bile acid sequestrant chenodeoxycholic acid (112). Thus, induction of OATP1B3 by FXR is another example of a responsive bile acid transport system that is decreased in conditions of low bile acid concentrations. It is notable that bile acid concentrations are decreased in conditions of bile acid deficiency due to the treatment of cholestatic diseases with cholestyramine or the bile acid sequestrant chenodeoxycholic acid (112). Thus, induction of OATP1B3 by FXR is another example of a responsive bile acid transport system that is decreased in conditions of low bile acid concentrations. It is notable that bile acid concentrations are decreased in conditions of bile acid deficiency due to the treatment of cholestatic diseases with cholestyramine or the bile acid sequestrant chenodeoxycholic acid (112). Thus, induction of OATP1B3 by FXR is another example of a responsive bile acid transport system that is decreased in conditions of low bile acid concentrations. It is notable that bile acid concentrations are decreased in conditions of bile acid deficiency due to the treatment of cholestatic diseases with cholestyramine or the bile acid sequestrant chenodeoxycholic acid (112). Thus, induction of OATP1B3 by FXR is another example of a responsive bile acid transport system that is decreased in conditions of low bile acid concentrations. It is notable that bile acid concentrations are decreased in conditions of bile acid deficiency due to the treatment of cholestatic diseases with cholestyramine or the bile acid sequestrant chenodeoxycholic acid (112). Thus, induction of OATP1B3 by FXR is another example of a responsive bile acid transport system that is decreased in conditions of low bile acid concentrations. It is notable that bile acid concentrations are decreased in conditions of bile acid deficiency due to the treatment of cholestatic diseases with cholestyramine or the bile acid sequestrant chenodeoxycholic acid (112). Thus, induction of OATP1B3 by FXR is another example of a responsive bile acid transport system that is decreased in conditions of low bile acid concentrations.
The Na+-taurocholate cotransporting 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 (22, 27, 112). Thus, in addition to enhancing bile acid efflux transport of cultured cells with bile acids (75). Two molecular mechanisms, potentially operational in parallel, have been proposed for this phenomenon: it has been suggested that SHP interferes with RAR-RXR-dependent transactivation (75) or GR-dependent transactivation (19) of the human ASBT promoter. Given that the FXR-SHP pathway also negatively targets GR on the human NTCP promoter, the latter...
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proposed pathway emphasizes the coordinated regu-
lation 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 choles-
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 ATPC 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 transcription factors are the monomeric nuclear receptor liver receptor homolog-1 (LRH-1) and homodimeric HNF-4α, which are overlapping DNA-binding motifs on both pro-
moters. 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 LRH-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 fibroblast growth factor-19 (FGF19; mouse ortholog Fgfg15) in the intestine (33, 38). From the intestine, 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 the fibroblast growth factor receptor-4 (FGFR4) DNA-binding domain of FXR, also leads to a reduction 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 syn-
thesis, since the effect seems clearly attenuated in SHp-
deficient mice (38). The FGF19/Fgf15-mediated endocrine-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 polymorphisms and mutations in several members of the nuclear receptor family, such as per-
some 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 approximate-
ly 400 genetic single nucleotide polymorphisms (SNPs) or mutations within the NR1H4 gene encoding FGF19/Fgf15 in the human or chimpanzee genomes, most of them located in intronic regions or in regions flanking the FGF19/Fgf15 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 cholestasis, which is frequently associated with adverse pregnancy outcomes, such as premature birth, fetal distress, and intra-uterine death. Four hetero-
zygous NR1H4 variants were identified: –1G>T substitution in the base position
–1G>T and 1A>G (M1V), 238T>C (W80R), and 518T>C (M173T). The first two FNR variants, –1G>T and 1A>G (M1V), were shown to lead to reduced FXR protein expression and decreased level of transactivation of a FXR-
dependent promoter construct in transfected human embryonic kidney HEK293T cells compared with the wild-type FNR. The 518T>C (M173T) variant, harboring an amino acid substitution within the zinc finger DNA-binding domain of FNR, 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 apparent-
ly 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 transcriptional initiation site. Although in this study the polymorphism was present in all ethnic groups and appeared in a similar frequency in controls and affected cases, it may still be due to chance. Interestingly, target genes with reduced transcript levels in the presence of the 1G>T substitution were suggested to be involved in the intrahepatic cholestasis of pregnancy.

In the study by van Mil et al. (97), a case-control study was performed in 70 ICP cases, 290 non-ICP cases, 59 controls, and 24 familial ICP cases. The identified genetic variant was not expressed in the control group but was present in a subset of ICP cases and the familial ICP cases. Another group published simultaneous reports on genetic 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 transcriptional initiation site. Although in

Table 2. Intestinal 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>CYP7A1</td>
<td>+</td>
<td>Heterodimeric basolateral bile acid efflux system</td>
</tr>
<tr>
<td>ASBT</td>
<td>–</td>
<td>Apical bile acid uptake system</td>
</tr>
<tr>
<td>FGF19</td>
<td>+</td>
<td>Bile acid-inducible growth factor</td>
</tr>
<tr>
<td>iNOS</td>
<td>+</td>
<td>Nitric oxide synthase, anti-microbial</td>
</tr>
<tr>
<td>angiotensin</td>
<td>+</td>
<td>Anti-microbial</td>
</tr>
</tbody>
</table>

For details and references to literature, please see the main text. +, induced by FXR; -, suppressed by FXR.

Downloaded from http://physiologyonline.physiology.org/ by 10.23.04.18 on July 6, 2017
d and the codon 80ile-preferred variant 238T>C (W80R) was not present in subjects of Caucauan origin, and only one British ICP patient carried the 1A>G (M1V) variant. Out of the two variants present in both the ICP group and the control group, 1G>T and 1H7–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 the ICP phenotype in the Swedish group. 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 action was abolished in FXR-null mice, indicating that this factor may have a role in the development of gallstone disease. In another report (9), the zinc finger protein FIC1 acts as an aminophospholipid flippase, transporting phosphatidylethanolamine across the absorptive and canalicular membrane and at the apical membrane of enteroocytes, in addition to many other tissues (94).

**Cholesterol cholelithiasis**

Having previously identified the N N4h gene as a candidate gene for the cholesterol gallstone susceptibility locus on 10q17 in mice (103), Kovacs et al. (54) recently proceeded to genotype the human NR1H4 gene in 481 gallstone carriers and 523 control individuals from three different ethnic populations. While no polymorphisms leading to an amino acid change were identified in this study, the haplotype termed NR1H4_1C, containing the more frequent allele variants in three base positions (–20647T>G and –1G>T in the 5′ region of the gene, and IVS7–31A>T within intron 7), was associated with gallstone prevalence in the Mexican male group but not in the German or Chilean population. It will be interesting to establish whether these haplotypes lead to altered levels of expression of FXR in the liver and in the intestine, thus affecting the amount of cholesterol available for biliary excretion. No significant difference in FXR mRNA levels was reported in liver biopsies obtained from 11 untreated cholesterol cholelithiasis patients when compared with 9 cholesterol gallstone-free subjects (6). However, in this latter study, the allelic status of the NR1H4 genes was not determined in the patients. Although the expression of FXR itself was not altered in this group of gallstone patients, the transcriptional coactivator 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 PPARα and HNF-1α (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.

Furthermore, the coding region of the NR1H4 gene is expressed at the liver whereas the FXR mRNA expression remains comparable, further indicating that this polymorphism may lead to compromised function but not expression level of FXR (6).

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 significantly less expressed in the livers of patients with cholesterol cholelithiasis when compared with 9 cholesterol gallstone-free subjects (6). However, in this latter study, the allelic status of the NR1H4 genes was not determined in the patients. Although the expression of FXR itself was not altered in this group of gallstone patients, the transcriptional coactivator 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 PPARα and HNF-1α (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.

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plasma membrane. Genetic mutations and polymorphisms in the ATP8B1 gene have been associated with familial intrahepatic cholestasis, characterized by low γ-glutamyltransferase plasma levels (reviewed in Refs. 41, 96). A disease entity termed progressive familial intrahepatic cholestasis type 1, or PFIC1 (also known as “Bolder’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 known 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 variants in the ATP8B1 gene, and it could be hypothesized that the severity of the disease may correlate with the degree of corresponding effect in FIC1 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 FIC1 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 BSEP promoter, the PFIC1-associated FIC1 variants were inactive, and the BRIC1-associated FIC1 variants activated the BSEP 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 targetting FXR and that FIC1-related disease may be caused by the compromised ability of the associated FIC1 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 prevent systemic infections caused by bacterial translocation 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 those 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 angiogenin, which also exerts anti-bacterial effects (34). It may be that agonistic ligands for FXR could be therapeutically useful in patients with reduced bile flow and consequently elevated bacterial growth and invasion across the intestinal 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 consequences of FXR activation, and thus differential physiological downstream effects. Furthermore, since FXR-null mice have been devoid of any FXR function function, 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 BSEP promoter, the PFIC1-associated FIC1 variants were inactive, and the BRIC1-associated FIC1 variants activated the BSEP 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 targetting FXR and that FIC1-related disease may be caused by the compromised ability of the associated FIC1 variants to influence FXR localization and function.


The bile acid receptor FXR regulates many aspects of bile acid homeostasis. FXR antagonists include the plant sterols stigmast-5-en-3β-ol (stigmasterol) and sitostanol. Early in the 20th century, it was reported that stigmasterol and sitostanol prevented cholesterol gallstone formation. The molecular mechanisms underlying this effect are not fully understood. However, recent studies have shown that these sterols activate the FXR-dependent expression of several hepatic genes involved in bile acid and cholesterol metabolism. These findings have led to the development of FXR agonists as potential treatments for various diseases, including obesity, type 2 diabetes, and cancer. Further research is needed to fully elucidate the role of FXR in bile acid and cholesterol metabolism and to identify new targets for the development of novel therapeutic agents.