Chapter 5

Identification of a new FXR target gene: fibrinogen Bβ

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5.1 Abstract

Bile salts act as detergents and keep hydrophobic compounds in solution in the gastrointestinal tract. They are also potent regulators of gene transcription. They bind and activate the Farnesoid X Receptor (FXR), which regulates transcription of genes encoding enzymes and transmembrane transporters involved in bile salt and cholesterol metabolism. FXR is also known to regulate bile salt/cholesterol-unrelated genes. In this study, we performed a transcriptome analysis to determine the genome-wide effect of FXR-activation and to identify possible new FXR target genes. Native and ratFxr-overexpressing HepG2-rNtcp cells were cultured in the absence or presence of the bile salt chenodeoxycholic acid. Changes in mRNA levels were analyzed using microarrays and real time RT-PCR.

Besides known non-bile salt/cholesterol-related FXR-target genes like kininogen, apolipoprotein AV, apolipoprotein C-III and SLC21A88, Fibrinogen Bβ was identified as a novel gene positively regulated by FXR and chenodeoxycholic acid. Analysis of the 5′ regions of the human, mouse, and rat Fibrinogen Bβ gene revealed a highly conserved inverted repeat element separated by 9 nucleotides that might be involved in the FXR-dependent regulation.

In conclusion, this study reveals a novel, positively regulated FXR-target gene, Fibrinogen Bβ, which encodes for a plasma glycoprotein that is involved in blood clotting.
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5.2 Introduction

Bile salts are the main solutes in bile and function as stimulators of fat digestion. They also facilitate the biliary secretion of hydrophobic compounds such as cholesterol and toxins. In recent years, bile salts have been shown to perform many other physiological functions. For example, they are mediators of apoptosis, activators of protein kinase C and phosphatidylinositol-3 kinase. Most importantly, bile salts play a crucial role as regulators of gene transcription, by means of binding and activation of the transcription factor, farnesoid X receptor (FXR).

FXR belongs to the nuclear hormone receptor superfamily of ligand-regulated transcription factors. It is active as a heterodimer with the retinoic acid receptor (RXRα). RXRα is activated by the vitamin A derivative 9-cis retinoic acid. The activated FXR/RXR heterodimer binds to a specific DNA sequence, the FXR responsive element (FXRE), in the promoter region of target genes. This FXRE consists of an inverted repeat element of two 6 base pair motif, AGGTCA, with a 1-base pair spacing (IR-1). FXR was first identified as a receptor for farnesol metabolites, and is expressed in liver, gut, adrenal gland and kidney. Recent data show that this nuclear receptor is the mammalian bile salt sensor that plays an important role in maintaining bile salt and cholesterol homeostasis. In accordance with this role, several FXR-target genes have been identified that are involved in bile salt and cholesterol metabolism. These include (1) the bile salt export pump (BSEP, ABCB11), the major hepatic bile salt exporter; (2) the intestinal bile acid-binding protein, which is an intestinal protein that binds bile salts with high affinity in the cytosol of enterocytes and (3) the transcription factor small heterodimer partner 1. Small heterodimer partner 1 in its turn represses transcription of cytochrome P450 7a1, the rate-limiting enzyme of bile salt synthesis and the sodium-dependent taurocholate cotransporting protein (Ntcp), the major hepatic bile salt importer. apolipoprotein A-I and the phospholipid transfer protein are both involved in HDL-cholesterol metabolism and are also regulated by FXR.

Recent studies have shown that FXR is also involved in the transcription of non-bile salt and cholesterol-related genes. These include apolipoprotein A1 and apolipoprotein C-II, which are both involved in triglyceride metabolism. Also the organic anion transporting polypeptide 8 (OATP8 or SLC21A8), transporter of organic anions and xenobiotics, and Kininogen (KNG), which is involved in anti-coagulation fall into this latter category. KNG transcription shows the most pronounced response to CDCA-activated FXR of all FXR-target genes studied thus far.

In the present study, we performed a transcriptome analysis on human HepG2 cells stably expressing rat Fxr to determine the effect of activated Fxr on approximately 18,000 human genes with known functions. In addition to the known FXR target genes indicated above, we show that also Fibrinogen Bβ (FGB) gene transcription is positively regulated by bile salts and FXR. The genes for human, rat and mouse FGB contain a sequence in their promoter elements that closely resembles a FXRE. However, in contrast to the IR-1, the inverted repeat sequences in the FGB promoter elements are separated by 9 nucleotides. This IR-9 sequence might be a novel FXRE involved in FXR-dependent regulation of FGB. These results provide evidence for an
increasing number of genes unrelated to bile salt or cholesterol metabolism that are regulated by FXR. Since FXR is considered a drug target for the treatment of cholestatic conditions, possible unwanted effects may result from the changed expression of these other FXR-target genes.

5.3 Materials and methods

Cell culture
The stably transfected human hepatoma cell line HepG2, expressing the rat sodium-dependent taurocholate cotransporting protein (HepG2-rNtcp) were cultured at 37°C in a humidified atmosphere of 5% CO₂ in air, in DMEM with GlutaMAX-1, 4500 mg/L D-glucose, sodium pyruvate, pyridoxine supplemented with 10% (vol/vol) heat-inactivated FBS, 100 U/ml penicillin G, 100 µg/ml streptomycin, 250 ng/ml fungizone and 250 µg/ml genetricin (Invitrogen BV, Breda, The Netherlands). The cell culture was passed twice a week.

Stable transfection
Plasmid DNA was isolated and purified using the EndoFree® Plasmid Maxi Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer’s instructions. HepG2-rNtcp cells were transfected by the calcium phosphate co-precipitation method using an expression vector for rat Fxr, pCMXrFxr and the hygromycin B-selection plasmid, pHMR272. Upon selection on hygromycin B-resistance, the rFxr-expressing cells were further cultured in normal HepG2-rNtcp medium.

Microarray
For the microarray analysis, HepG2-rNtcp and HepG2-rNtcp-rFxr cells were cultured in the presence or absence of 100 µmol/L CDCA (sodium salt, Calbiochem-Novabiochem, San Diego, CA) dissolved in phosphate-buffered saline (PBS) for three days. Total RNA was isolated as described and labeled using the Agilent Fluorescent Direct Label Kit according to the manufacturer’s instructions (Agilent Technologies, Palo Alto, CA). The control samples (HepG2-rNtcp+PBS) were labeled with Cyanine-5 and the experimental samples (HepG2-rNtcp+CDCA and HepG2-rNtcp-rFxr + CDCA) were labeled with Cyanine-3. The labeled cDNA’s were hybridized on Human 1A Oligo Arrays, using the Agilent Oligonucleotide Microarray Hybridization Kit (Agilent Technologies, Palo Alto, CA). The experiment was done in duplo. The arrays were collected and analyzed using the Agilent Microarray Scanner System and Feature Extraction Software (Agilent Technologies, Palo Alto, CA). Genes meeting the criteria (average of cyanine-3 and cyanine-5 normalized signals > 8 and P-value of the Log Ratio < 0.01) were used for further analysis.

Reverse-transcription polymerase chain reaction (RT-PCR)
Total RNA isolation and quantitative real-time detection RT-PCR analysis of BSEP/Bsep, FGB/Fgb, SLC21A8, and KNG mRNA levels were described before. Details about primer- and probe-sequences are available at the authors.
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Animals
Male C57BL/6J mice (Harlan Nederland, Horst, the Netherlands) were housed in a temperature-controlled environment with alternating 12 hours light and dark cycles. Food and water were available ad libitum. Experimental protocols were approved by the Ethical Committee on Animal Testing of the Faculty of Medical Sciences, University of Groningen.

Experimental design
At the age of 13 weeks, the mice were divided into groups. Group 1 continued with their normal diet, while group 2 received their diet supplemented with 0.5% (wt/wt) cholic acid (CA). One week later, the mice were weighed and killed. The livers were removed, weighed, cut into pieces, snap-frozen in liquid nitrogen, and stored at -80°C for mRNA isolation.

Statistic
Data are presented as means ± sd. Differences between the two animal groups were determined in SPSS by a Mann-Whitney U-test and p<0.05.

5.4 Results
Generation of rFxr-expressing HepG2-rNtcp cells
FXR is a potent regulator of transcription of genes involved in bile salt and cholesterol metabolism. As such, it is regarded an important target for controlling bile salt/cholesterol homeostasis in humans.

![Diagram showing relative BSEP mRNA levels](image)

Fig. 5-1 CDCA-induced BSEP expression is strongly increased in HepG2-rNtcp cells stably transfected with rat Fxr. HepG2-rNtcp cells were incubated with vehicle (PBS, black bar) or 100 µM CDCA (gray bar) and HepG2-rNtcp-rFxr cells were incubated with 100 µM CDCA (white bar). Total RNA was isolated and BSEP mRNA levels were determined by real time RT-PCR. The level of 18S was determined as control.
In recent years, novel FXR-target genes have been identified that are not involved in bile salt/cholesterol metabolism. These genes may cause unwanted or unexpected side effects during FXR-targeted therapy. We therefore determined the effect of activated FXR on the transcription of approximately 18,000 human genes with the aim to characterize FXR-controlled processes unrelated to bile salt/cholesterol homeostasis. We generated HepG2-rNtcp cells that stably express rat Fxr for the identification of new FXR- and CDCA-regulated genes. BSEP mRNA expression served as a positive control for the functionality of rFxr in this cell line. Both native and rFxr-expressing cells were treated with 100 µM CDCA and compared to the native cells treated with vehicle. As expected, BSEP expression was significantly increased by both CDCA and rFxr, showing 7-fold increased mRNA levels in CDCA-treated HepG2-rNtcp and a 68-fold increase in CDCA-treated HepG2-rNtcp-rFxr cells, respectively, compared to HepG2-rNtcp cells grown in the absence of CDCA (Fig. 5-1).

Selection of significant up-regulation or down-regulation of genes in microarray

Next, the same cDNA samples were analyzed using the Agilent Human 1A Oligo Arrays to identify novel FXR target genes. Four individual microarray hybridization experiments were performed. cDNA from control cells (group C) were labeled with Cy5 and used as control in all 4 experiments. cDNA from CDCA-treated HepG2-rNtcp (CDCA) or HepG2-rNtcp-rFxr (FXR/CDCA) cells were labeled with Cy3. Two arrays were hybridized with the combination C versus CDCA (chip 1a and b). The other two with the combination C versus FXR/CDCA (chip 2a and b). The rational behind this setup was that possible rFxr-unrelated CDCA effects on transcription could be excluded, and that limited regulation of FXR-target genes would be strongly enhanced in the rFxr-transfected cells. Criteria were set to evaluate the significance of changed expression levels under the 3 different conditions. These parameters were: (1) The CDCA-induced expression (C versus CDCA) should be at least 1.5; (2) the average of both (Cy3 and Cy5) normalized (log) signal intensities should be > 8. This limit is arbitrary and signals with an intensity of ≤ 8 are assumed to be unreliable; (3) the P value for significance was < 0.01. Four hundred thirty five (435) transcripts met these criteria on both a and b chips and were included for the final analysis: The rFxr-induced expression (CDCA versus FXR/CDCA) should be at least 1.2.

Positively regulated genes by CDCA-activated rFxr

BSEP expression, though used to validate our experimental setup (Fig. 5-1), did not meet the microarray analysis parameters. This was, however, not unexpected since HepG2 cells are well known to express BSEP only at very low levels. Even the 68-fold increase of BSEP in the CDCA-treated HepG2-rNtcp-rFxr cells remained below the detection limits of the microarray 2. Two other established FXR-target genes, SLC21A8 and KNG, were expressed to significant levels and were selected to determine their expression differences between the two arrays (bold in Table 5-1). KNG shows the strongest relative increase to CDCA-treatment, i.e. 5.5-fold in
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HepG2-rNtcp cells and 7.9-fold in HepG2-rNtcp-rFxr cells. SLC21A8 mRNA expression levels were 2.2-fold increased in HepG2-rNtcp cells and 3.0-fold in HepG2-rNtcp-rFxr cells. The additive effect of rFxr for both SLC21A8 and KNG, is a 1.4-fold increase according to microarray analysis.

<table>
<thead>
<tr>
<th>Name</th>
<th>CDCA mean±sd</th>
<th>FXR/CDCA mean±sd</th>
<th>CDCA vs. FXR/CDCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGB</td>
<td>2.68±0.87</td>
<td>5.72±2.02</td>
<td>2.1</td>
</tr>
<tr>
<td>KNG</td>
<td>5.49±2.06</td>
<td>7.91±2.50</td>
<td>1.4</td>
</tr>
<tr>
<td>FGG</td>
<td>1.99±0.22</td>
<td>2.79±0.49</td>
<td>1.4</td>
</tr>
<tr>
<td>SLC21A8</td>
<td>2.16±0.33</td>
<td>2.98±0.78</td>
<td>1.4</td>
</tr>
<tr>
<td>SURF6</td>
<td>2.07±0.79</td>
<td>2.77±0.16</td>
<td>1.3</td>
</tr>
<tr>
<td>FGA</td>
<td>1.41</td>
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<td>1.3</td>
</tr>
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<td>3.01±0.05</td>
<td>1.3</td>
</tr>
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</tr>
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<td>1.3</td>
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<td>TMOD4</td>
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<td>I_1152006</td>
<td>2.91±0.06</td>
<td>3.56±0.04</td>
<td>1.2</td>
</tr>
<tr>
<td>CAPN5</td>
<td>3.10±0.42</td>
<td>3.77±0.17</td>
<td>1.2</td>
</tr>
<tr>
<td>PAP2A</td>
<td>3.33±0.54</td>
<td>4.05±0.26</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Table 5-1: List of human genes of which the expression is increased at least 1.2-fold by CDCA and rFXR in HepG2-rNtcp cells. Agilent Human 1A Oligo arrays were hybridized with cDNA obtained from HepG2-rNtcp cells and HepG2-rNtcp-rFXR cells treated with CDCA (CDCA and FXR/CDCA respectively). The mRNA expression levels of both CDCA-treated cell lines were compared to those in HepG2-rNtcp cells treated with the vehicle, PBS (group C). Only the genes that meet the criteria as described in experimental procedures were analyzed for the effect of the presence of rFXR. Note: mRNA expression levels for FGA in CDCA column did not meet all criteria, but are included for comparison to FGB and FGG.

To more accurately quantify the relative increase caused by CDCA and rFXR separately, RT-PCR was performed for SLC21A8 (Fig. 5-2a) and KNG (Fig. 5-2b). In CDCA-treated HepG2-rNtcp cells, the SLC21A8 and KNG mRNA levels were increased with 4.9-fold and 20.6-fold, respectively. These values further increased to 10.2-fold and 33.7-fold, respectively, in the CDCA-treated HepG2-rNtcp-rFXR cells. According to this analysis the rFXR-specific effect for SLC21A8 is 2.0-fold and for KNG 1.6-fold, whereas for BSEP a rFXR effect of 9.7 was detected (Fig. 5-1).
Fig. 5-2 Human FGB expression is increased by CDCA and rFxr in HepG2-Ntcp cells. HepG2-rNtcp cells were incubated with vehicle (PBS, black bar) or 100 µM CDCA (gray bar) and HepG2-rNtcp-rFxr cells were incubated with 100 µM CDCA (white bar). Total RNA was isolated and SLC21A8 (a), KNG (b), and FGB (c) mRNA levels were determined by real time RT-PCR. The level of 18S was determined as control.

**Human Fibrinogen Bβ is a putative FXR-target gene**

Table 5-1 shows that only few additional genes confirmed to our set criteria of 1.5-fold up-regulation upon CDCA-treatment of HepG2-rNtcp cells and a further significant increase of at least 1.2-fold in CDCA-treated HepG2-rNtcp-rFxr cells. The most eye-catching genes from this shortlist are that of Fibrinogen Aα, Bβ and gamma (FGA, FGB and FGG). Together, these genes encode the plasma protein Fibrinogen. The FGB gene shows the strongest response to rFxr from all detected genes in the microarray experiment. The FGB mRNA was increased 2.7-fold and 5.7-fold in CDCA-treated HepG2-Ntcp and HepG2-Ntcp-Fxr cells, respectively. Thus, the rFxr-effect for FGB amounts to 2.1-fold. Though significantly below the 9.7-fold observed for BSEP by RT-PCR, it tops all genes detected in the microarray experiment, including KNG and SLC21A88. The rFxr-effect was confirmed by RT-PCR analysis.
for FGB, in which a 2.7-fold and 6.0-fold increase was detected in CDCA-treated HepG2-Ntcp and HepG2-Ntcp-Fxr cells, respectively. In addition, the expression of FGA and FGG was also significantly increased by CDCA and rFxr (Table 5-1). For FGG the CDCA-effect was 2.0-fold and the additional rFxr-effect 1.4-fold. Although FGA (in italic in table 5-1) did not meet our preset criterion of 1.5 (the CDCA-effect was just 1.4-fold), we added this gene to the list of up-regulated genes, because of its relation to FGB. The additional rFxr effect for FGA was 1.3-fold. Thus, all three genes encoding the 3 subunits of Fibrinogen show enhanced expression upon CDCA-activation of rFxr in HepG2-Ntcp cells.

**Fibrinogen Bβ mRNA expression in cholate-fed mice**

To determine whether FXR-controlled expression of FGB also occurs in vivo we studied hepatic expression in mice fed a bile salt-containing diet and compared these to mice fed normal chow. Male C57BL/6J mice were fed a 0.5% cholate diet during one week. Total RNA from the livers was isolated and Fgb and Bsep mRNA expression levels were quantified using RT-PCR (Fig. 5-3). The Fgb mRNA level was 1.3-fold (p = 0.073) increased in cholate-fed animals compared to control mice, whereas Bsep mRNA levels were 1.7-fold (p = 0.001) higher in the cholate-fed animals.

![Fig. 5-3](image_url) The effect of cholate-feeding on hepatic Fgb expression in mouse. C57BL/6J mice were fed either a control diet (black bars) or a 0.5% CA-supplemented diet (white bar) for one week. Total RNA was isolated from their livers and Bsep and Fgb mRNA levels were determined by real time RT-PCR. The level of 18S was determined as control. Data are presented as mean ± sd. (*: significantly different from control diet, p < 0.05)

**Identification of a putative FXRE in the human Fibrinogen Bβ promoter region**

The positive regulation of the FGB gene by CDCA and rFxr suggest that FXR is directly involved in the regulation of this gene. This implies that FXR binds the FGB promoter element. Concurrently, we screened the FGB promoter region from human (gi:31400), mouse (gi:38077445) and rat (gi:204100) for candidate FXRE’s. All three sequences show a high degree of identity/similarity in the region immediately upstream of the transcription start site (Fig. 5-4). In this region we identified a highly conserved element (from nucleotide –94 to –74 in the human FGB promoter; +1 is
the transcription initiation site), which may function as an FXRE. In human and rat, it consists of a perfect inverted repeat of two 6 base pair-motifs, GGTTCA and GTAACC, which are separated by 9 nucleotides (which would then be an IR-9). In the 5'-flanking region of the mouse Fgb gene, there is one nucleotide difference compared to the human and rat sequence; ΔGGTTCA and ΔGTAACC.

**Fig. 5-4** The promoter region of mammalian FGB genes contain a putative FXRE. Sequences of the 5'-flanking regions of the human, mouse, and rat FGB genes are shown. The conserved nucleotides are indicated by *. The site of transcription initiation is designated as +1 (Δ). The regulatory sequences IL-6RE, C/EBP, HNF1, and TATA-box, established by previous studies, are underlined. The proposed IR-9 is boxed, the inverted repeat element is shown in bold.

### 5.5 Discussion

In this study we show that Farnesoid X receptor (FXR) directly regulates the transcription of genes encoding human Fibrinogen. This is another example of FXR regulating the transcription of genes that appear unrelated to bile salt and/or cholesterol homeostasis. These effects need to be considered in future therapies aiming at manipulating bile salt and/or cholesterol levels through FXR.

FXR is generally viewed as the bile salt sensor that regulates transcription of key genes involved in bile salt homeostasis. It may activate transcription of target genes directly after binding to FXR-response elements in their promoter region, or repress transcription indirectly through up-regulation of the transcriptional repressor, small heterodimer partner (SHP-1). Genes involved in bile salt homeostasis that are directly or indirectly regulated by FXR include the ones for the rate-limiting enzyme in bile salt biosynthesis, cytochrome p450 7a1, the hepatic bile salt transporters Ntcp (uptake) and BSEP (secretion), and intestinal bile acid binding protein. It is therefore an important pharmacological target to control the enterobacterial circulation of bile salts. Recent studies have shown that FXR also directly regulates genes that are not linked to bile salt and cholesterol metabolism. We performed microarray analyses to reveal more of these non-bile salt and cholesterol related genes as targets for FXR. HepG2-rNtcp cells and their rat Fxr-expressing derivatives, the HepG2-rNtcp-rFxr cells, were
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treated with CDCA. This treatment resulted in increased mRNA expression levels of already established FXR-regulated genes, like $SLC21A8$ and $KNG$. $BSEP$ expression remained undetectable in the microarray experiments, even though its expression is 68-fold increased in the CDCA treated HepG2-rNtcp-rFxr cells. $BSEP$ expression is known to be low in HepG2 cells. The limited sensitivity of microarray experiments is clearly a drawback of this technique and biases the results towards highly expressed genes. Physiological important FXR-target genes may therefore escape identification using immortalized cells like HepG2 that show strongly reduced basal mRNA levels of subsets of genes, including $BSEP$ and $NTCP$. However their accessibility towards genetic manipulation, here stable rFxr-overexpression, does result in relevant data about genes that are (still) expressed at detectable levels in such cells. The gene that showed the highest increase upon FXR-activation was $Fibrinogen B_\beta$ ($FGB$). $FGB$ is a subunit of Fibrinogen that in addition consists of Fibrinogen $\alpha$ ($FGA$) and $\gamma$ ($FGG$). Fibrinogen is a plasma glycoprotein synthesized in the liver and plays a role in blood clotting. The $FGA$, $FGB$ and $FGG$ genes are located in a cluster on chromosome 4. $^{24}$ Upon vascular injury, it is cleaved by thrombin to form fibrin. This is achieved by coordinate transcriptional regulation of all three genes, in which interleukin-6 (IL-6) plays an important role. $^{25}$ In addition, several cleavage products of fibrinogen and fibrin are involved in cell adhesion, vasoconstriction, chemotactic and mitogenic activities. $^{26}$ The promoter regions of the human, mouse and rat $FGB$ genes are highly conserved between position $-157$ and position $+17$ relative to transcriptional start site. Previous studies have shown that several regulatory elements are located within this region. $^{27}$ For example, the relevance of an IL-6 responsive element, a C/EBP motif and an HNF-1 binding site has been demonstrated. $^{26,30}$ Our analysis of this region revealed a highly conserved and new element between nucleotides $-94$ and $-74$. This element consists of a perfect inverted repeat separated by 9 base pairs (GGTTCA-N₉-GTAACC). We hypothesize that this inverted repeat may be a novel FXR responsive element. The consensus FXRE is an inverted repeat of the AGGTCA motif with a 1 base pair spacing (IR-1). However, many exceptions on this consensus have been described that also bind FXR, resulting in increased transcription of the downstream gene. The rat dehydroepiandrosterone sulfotransferase promoter contains an inverted repeat with no spacing (IR-0), the human multidrug resistance-related protein 2 promoter contains an everted repeat with a 8 base pair spacing (ER-8), and human apolipoprotein-AV contains an inverted repeat with an 8 base pair spacing. $^{15}$ Since the IR-9 in the $FGB$ promoter is conserved between species, we consider this sequence as a putative FXRE. A previous study already identified the positions $-94$ and $-93$ in the human $FGB$ promoter as a positive cis-acting element. $^{30}$ The IR-9 is present in the 5′-flanking region of both rat and mouse $Fgb$. Therefore, we performed experiments with freshly isolated primary rat hepatocytes, exposed them to CDCA and determined the effect of $Fgb$ gene transcription. Unfortunately, $Fgb$ mRNA levels strongly increase during the isolation procedure of hepatocytes, which returns to basal levels only 2 to 3 days after seeding the cells. During this period, expression of the bile salt uptake transporter, $NTCP$, is reduced to below 10% of its normal level in hepatocytes. Consequently, we have not been able to determine the effect of bile salts on the expression of $Fgb$ in rat hepatocytes. In addition, we have
analyzed the expression of Fgb in livers of mice fed a diet containing 0.5% cholate for 1 week. The Fgb mRNA expression showed a tendency to be increased in livers of the CA-fed animals (1.3-fold) compared to animals fed control chow, however the difference was not significant (P=0.073), primarily due to small number of animals analyzed (control diet: n=6; cholate diet n=7). The relatively low induction level of Fgb does, however, not mean that this might not be physiological relevant. Also expression of Bsep was only increased 1.7-fold and this has previously been shown to result in a significant increase of hepatic bile salt secretion.33

No candidate FXRE’s were detected in the promoter elements of the FGA and FGG genes even though the expression of both genes were increased by FXR and CDCA. The increased expression of FGA and FGG may however be caused by FGB, since it is known that overexpression of one of the Fibrinogen genes may stimulate expression of the other two genes.34

Cholestatic liver diseases, like primary biliary cirrhosis and primary sclerosing cholangitis, are known to be associated with preserved clothing function or even a hypercoagulable state.35,36 This may explain the high incidence of portal vein thrombosis in primary biliary cirrhosis.35 The hypercoagulable state may in part offset the decreased synthesis of clothing factors during advanced liver cirrhosis. The study of Pihusch et al., showed increased Fibrinogen levels in primary biliary cirrhosis and primary sclerosing cholangitis patients.36 Our findings suggest that in cholestatic liver diseases fibrinogen synthesis is stimulated by bile salts, thus counteracting a decreased synthesis due to cirrhosis. This may aid the preservation of blood clothing in cholestatic liver disease.

In conclusion, our results show that FGB transcription is positively regulated by FXR and bile salts. As such, it is another example showing that FXR transcriptional regulation is not restricted to genes involved in bile salt and cholesterol metabolism.

Note:
During finalizing this thesis, Anisfeld et al.37 also reported that FXR activates FGB gene transcription. Our results are largely in line with their study. In addition, they performed a promoter deletion analysis to locate the FXR response element (FXRE) in the human FGB promoter. They show that the region -2281/-1700 is required for FXR-induced transcription after activation by the synthetic ligand GW4064. This region does not contain a sequence that conforms to the currently known FXRE’s. Future experiments need to reveal which sequence in the FGB promoter directly binds FXR.

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References
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