INCREASED EXPRESSION OF LXRα, ABCG5, ABCG8 AND SRBI IN THE LIVER FROM NORMOLIPIDEMIC NONOBESE CHINESE GALLSTONE PATIENTS

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ABSTRACT

Cholesterol supersaturation of bile is one prerequisite for gallstone formation. In the present study on Chinese patients with gallstones, we investigated if this phenomenon was correlated with the hepatic expression of genes participating in the metabolism of cholesterol and bile acids. Twenty-two non-obese normolipidemic patients (female/male: 11/11) with gallstone were investigated with 13 age and body mass index matched gallstone-free controls (female/male: 10/3). The bile from the gallstone patients had higher cholesterol saturation than the controls. The mRNA levels of ABCG5/ABCG8, and LXRα in the gallstone patients were increased by 51%, 59% and 102% respectively, and significantly correlated with the molar % of biliary cholesterol and cholesterol saturation index. The mRNA and protein levels of the hepatic SRBI were increased, and a significant correlation was found between the protein levels and the cholesterol saturation index. No differences were recorded between the two groups concerning the hepatic synthesis of cholesterol, bile acids and esterification of cholesterol. Our results suggest that upregulation of ABCG5/ABCG8 in gallstone patients - possibly mediated by increased LXRα - may contribute to the cholesterol supersaturation of bile. Our data are consistent with the possibility that increased amounts of biliary cholesterol may originate from plasma HDL cholesterol by enhanced transfer via SRBI.

Supplementary key words: SRBI, HNF4A1, ACAT2, nuclear receptors, ATP binding cassette, bile acids, cholesterol esters

Abbreviations: ACAT: acyl-coenzyme A: cholesteryl acyltransferase; ABC: ATP binding cassette; apoA1: apolipoprotein A1; CYP7A1: cholesterol 7α-hydroxylase; CYP8B1: cholesterol
12α-hydroxylase; CYP27: cholesterol 27α-hydroxylase; FXR: farnesoid X receptor; HMGCR: 3-hydroxy-3-methylglutaryl coenzyme A reductase; HNF4A: hepatocyte nuclear factor 4α; LRP: LDL-receptor related protein; LXRα: liver X receptor α; NPC1L1: Niemann-Pick C1-like 1 protein; PGC1α: peroxisome proliferative activated receptor γ coactivator 1 α; PPPA: pyripyropene A; SHP: small heterodimer partner; SRBI: scavenger receptor B type I; SREBP: sterol regulatory element binding protein.
INTRODUCTION

Cholesterol gallstone disease is common in both the industrialized and developing countries (1, 2). In 1995, the Chinese National Survey reported that gallstone disease accounted for nearly 10% of all the diagnoses of patients hospitalized in surgical clinics and the majority of the gallstones were composed of cholesterol (2).

Gallstone disease can be viewed as the terminal outcome of different metabolic disorders, caused by diverse genetic and environmental factors. It is a multi-factorial disease and the causes of gallstones are heterogeneous and mostly intrahepatic. The critical element for gallstone formation is supersaturation of bile with cholesterol (3).

Early studies attempted to define enzymatic defects in the liver contributing to the cholesterol supersaturation of bile. The activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR; the rate-limiting enzyme for de novo synthesis of cholesterol) was shown to be increased in gallstone patients (4). Conversely, the activities of cholesterol 7α-hydroxylase (CYP7A1; the rate-limiting enzymes for bile acid synthesis), and acyl-coenzyme A: cholesteryl acyltransferase (ACAT; the enzyme catalyzing cholesterol esterification), were both decreased in gallstone patients (4, 5). However, larger studies on different populations could not confirm these results (6, 7). Recently, in a study in Chilean Hispanics and Mapuche Indians, a surrogate marker of bile acid synthesis was measured in the plasma of gallstone patients that suggested an increased CYP7A1 activity (8).

Identification and characterization of the ATP binding cassette (ABC) transporters for cholesterol, bile acids and phospholipids has brought new insights to our understanding of gallstone disease. ABCG5 and ABCG8 appear to function as a heterodimer for secretion of cholesterol into the bile canaliculus (9). As shown before in mice (10, 11), both the Abcg5 and
Abcg8 genes are targets for the liver X receptor α (LXRα) and could be induced by LXRα agonists. ABCB11 (12) (also known as BSEP, bile salt export pump) is a major bile acid transporter, and ABCB4 (also known as MDR3, multiple drug resistance transporter 3) is a phospholipid transporter. In animal models, either overexpression of Abcg5/Abcg8 (9) or depletion of Abcb11 (13) or Abcb4 (14) modify biliary lipid secretion, and in some cases, lead to supersaturation of bile with cholesterol. As with most of the studies performed in mice, the role of ABC transporters in the pathogenesis of cholesterol gallstones is not well understood in humans.

In this study, we attempted to identify some of the molecular defects in hepatic cholesterol and bile acid metabolism involved in the pathogenesis of cholesterol gallstone disease. We studied a group of normolipidemic, non-obese Chinese patients, who neither had diabetes mellitus nor signs of insulin resistance. Our present results suggest that in these humans, supersaturation of bile is associated with an increased expression of ABCG5/ABCG8 and LXRα. We also observed an increased expression of scavenger receptor B type I (SRBI), which mediates hepatic uptake of cholesterol from HDL (15) that is directed to biliary cholesterol secretion.
MATERIALS AND METHODS

Subjects

Twenty-two Chinese patients (11 females and 11 males) with cholesterol gallstone disease (GS) and 13 Chinese gallstone-free patients (GSF; 10 females and 3 males) were included in this study. The GS underwent open or laparoscopic cholecystectomy. Gallbladder size was evaluated by ultrasonography and gallbladder function was assessed by analysis of total biliary lipids. Cholesterol gallstones were confirmed by visual inspection of the typical cut-surface of gallstone, or when necessary, by enzymatic cholesterol analysis. The GSF included 9 patients with gallbladder polyps (7 females and 2 males) undergoing cholecystectomy, and 4 patients treated with liver resection because of right hepatic hemangioma (3 females and 1 male). No gallstones were found in any of these controls after resection of the gallbladder, nor were cholesterol crystals found in bile by polarized light microscopy. The pathological examination of the polyps showed an inflammatory nature. None of the patients had any other disorders affecting the hepatic, gastrointestinal, renal and endocrine functions (i.e. either diabetes mellitus or signs of insulin resistance). The patients were not subjected to lipid-lowering treatment. Prior to enrollment in the study, informed consent to participate in the study and to collection of a liver biopsy was obtained. The study protocol was approved by the Ethics Committee both at the Ruijin Hospital, Medical School of Shanghai Jiaotong University, Shanghai, China and the Karolinska University Hospital at Huddinge, Stockholm, Sweden.

Procedure of sample collection

Patients were fasted overnight prior to the surgery, which was performed between 9 and 10 AM. After opening the abdomen, or following the application of pneumoperitoneum, a wedge biopsy of about 0.5-1.0 g was taken from the right lobe of the liver, snap-frozen in liquid nitrogen, and
stored at -70°C. Criteria for a functioning gallbladder consisted of: i) presence of dark concentrated bile in the gallbladder; ii) no evidence of impacted stones in the neck of the cystic duct at operation. After clamping the cystic duct, bile from the gallbladder was obtained by aspiration. All the cholecystectomies were performed without any complications. The participation in the study did not result in prolonged hospitalization and no serious adverse events were reported.

**Analysis of plasma lipids and lathosterol**

Plasma total cholesterol, triglycerides, HDL cholesterol, apo AI and apo B were analyzed by automated bioanalyzer (Roche Hitachi Modular P800, Japan) and corrected by the dilution due to addition of EDTA (2%). LDL cholesterol in plasma was calculated according to Friedewald’s equation. Lipoproteins were separated by size exclusion chromatography as previously described (16).

**Analysis of biliary lipids and bile acid composition**

Biliary cholesterol, total bile acids and phospholipids in gallbladder bile were measured as previously described (17). Cholesterol saturation index (CSI) was calculated using Carey’s critical table (18).

**Analysis of liver lipids**

Crude liver homogenates were prepared as described previously (7) and extracted in chloroform:methanol, 2:1 (v/v). Hepatic cholesterol concentrations were then assayed by gas chromatography-mass spectrometry (7). Unesterified lathosterol was determined by isotope dilution-mass spectrometry using a deuterium-labeled internal standard (19). Hepatic triglycerides were determined in liver lipid extracts by colorimetric enzymatic methods (TG
Roche/Hitachi, Roche Diagnostic GmbH, Mannheim, Germany). Protein content was determined according to Lowry’s method.

**Assay of microsomal ACAT1 and ACAT2 activity**

Total ACAT enzymatic activity was determined in hepatic microsomes, including a 30 minute preincubation with a cholesterol-saturated solution of β-hydroxypropyl cyclodextrin before addition of the 14C-oleoyl Co-A, as described (20). In a parallel incubation, pyripyropene A (PPPA), a specific ACAT2 inhibitor, was included in the preincubation and reaction mixtures at a concentration of 5 µmol/L in order to separately identify ACAT1 and ACAT2 activities (20).

**Relative RNA expression level measurements**

Hepatic total RNA was extracted with Trizol® (Invitrogen, Carlsbad, USA) and reversibly transcribed into cDNA (Omniscript, Qiagen Inc, Valencia, USA). Real-time quantitative PCR assays were performed in triplicate using SYBR-Green (MedProbe, Oslo, Norway). Primers (primer sequences are available on request) were designed using Primer Express 2.0, all with sequences crossing exon-exon boundaries. Data were calculated by the delta-Ct method, expressed in arbitrary units, and were normalized by the signals obtained from the same cDNA for Cyclophilin A. The fold change for each mRNA expression level in the GS was expressed relative to the obtained value of GSF, the mean value of which was arbitrarily set at 1.

**Western Blot analysis**

Twenty microgram of liver membranes from each patient sample were separated on 10% SDS-PAGE gel, and then transferred onto nitrocellulose membranes (Invitrogen, Carlsbad, USA). After blocking in 5% non-fat dry milk in PBST (0.05% Tween-20), the nitrocellulose membranes were incubated overnight at 4°C with rabbit anti-SRBI (1:3000; Abcam Ltd,
Cambridge, UK) in 5% non-fat milk powder in PBST. After washing with PBST, donkey anti-rabbit IgG F(ab’)2 antibody were added (1:50000; Amersham Bioscience AB, Uppsala, Sweden). The signals were detected using the SuperSignal chemiluminescence kit (Pierce Biotechnology Inc, Rockford, IL, USA) and a Fuji BAS 1800 analyzer (Fuji Photo Film Co., Japan) and quantified by the Image Gauge software (Science Lab, 98, version 3.12, Fuji Photo Film Co., Japan). After cleaning the membranes by stripping, they were further blotted with rabbit anti-β-actin (1:3000, Abcam Ltd, Cambridge, UK) as a loading control. Data are expressed as arbitrary units and normalized to the β-actin expression.

Statistics

Data are reported as mean ± SEM. Student’s t-test was used to compare the differences of variables between gallstone patients and gallstone-free controls (Statistica 7.0 software, StatSoft Inc, Tulsa, USA). Variables were correlated with Spearman’s Rank test. Statistical significance was set at $P < 0.05$. 
RESULTS

Clinic characteristics and plasma lipids

Demographic data for GS and GSF are shown in Table 1. No significant differences in age and BMI were observed. No differences in plasma lipids between GS and GSF were present (Table 1). Neither were there any significant differences in the distribution of cholesterol or triglycerides in the lipoprotein classes.

Biliary lipid composition and hepatic lipid content

Analysis of gallbladder bile was not possible for 8 patients due to technical problems during the surgical procedure. Furthermore, for ethical reasons, bile was not collected from 4 GSF who underwent surgery for removal of hepatic hemangioma. In the samples analyzed, a significantly higher molar percentage of cholesterol was present in the bile of the GS as compared to the GSF (Figure 1A), as well as a significantly higher cholesterol saturation index (GS vs. GSF: 1.04 ± 0.08 vs. 0.71 ± 0.05, P < 0.01). Neither the total bile acids nor the phospholipids in bile differed between the groups (Figure 1A). No differences were found in total biliary lipids (GS vs. GSF: 13.4 ± 1.26 g/dL vs. 13.5 ± 1.60 g/dL, P = NS), possibly because we made sure that all the GS patients had normal gallbladder function. The bile acid composition of the available gallbladder bile samples is shown in Figure 1B. In both groups, cholic acid and chenodeoxycholic acid comprised 70-80% of the total bile acids, without any differences between groups. Unexpectedly, in GSF, a significantly higher percentage of deoxycholic acid was found.

In the liver tissue, GS tended to have higher free cholesterol and lower cholesteryl ester concentrations than the GSF, but the differences were not statistically significant (Figure 1C). Similarly, no significant differences were observed for hepatic triglyceride (Figure 1D) and lathosterol concentrations (Figure 1E) between GS and GSF patients.
**ABCG5 and ABCG8 correlate with biliary cholesterol**

*ABCG5* and *ABCG8* mRNAs were significantly increased in GS compared with GSF (+51% for *ABCG5*, *P < 0.01* and +59% for *ABCG8*, *P < 0.01*, Figure 2A), and their mRNA levels correlated very well (*r* = 0.89; *P < 0.05, Figure 2B) confirming their likely co-expression in human liver *in vivo* (21). The *ABCG5* and *ABCG8* expression also correlated positively with the biliary cholesterol molar percentage (*r* = 0.57 and *r* = 0.54; *P < 0.05) and the CSI (*r* = 0.54 and *r* = 0.55, *P < 0.05).

Interestingly, the expression of *LXRα* was 102% higher in GS compared with GSF (*P < 0.01; Figure 2A). It correlated both with *ABCG5* (*r* = 0.58, *P < 0.05, Figure 2C) and *ABCG8* mRNA levels (*r* = 0.59, *P < 0.05), and also with the biliary cholesterol molar percentage (*r* = 0.45; *P < 0.05) and the CSI (*r* = 0.43 *P < 0.05, Figure 2D). In contrast, the expression of *ABCB11* and *ABCB4*, measured as mRNA abundance, did not differ significantly between GS and GSF, as was also the case for seventeen other genes involved in various other aspects of the regulation of hepatic lipid metabolism (Figure 2E).

**Bile supersaturation in cholesterol may originate from a SR-BI mediated pathway**

To further elucidate the possible mechanisms behind the bile cholesterol supersaturation and the increased expression of *ABCG5* and *ABCG8*, we examined genes considered to be key players in the regulation of the hepatic free cholesterol levels. The liver HDL receptor *SRBI* displayed 38% higher levels of mRNA in GS compared to GSF (*P < 0.05, Figure 2A). This was paralleled by a 74% increase of the *SRBI* protein (*P < 0.05, Figure 3A and 3B). The protein expression of SRBI significantly correlated with the mRNA levels (*r* = 0.46, *P < 0.05). In addition, the protein expression of SRBI correlated with both the molar percentage of biliary cholesterol (*r* = 0.56, *P < 0.05, Figure 3C) and the CSI (*r* = 0.52, *P < 0.05, Figure 3D). No differences were seen in the
mRNA of the genes for LDL-receptor, LDL-receptor related protein (LRP), apoA1, ABCA1, NPC1L1 and HMGCR (Figure 2E). Within the hepatocytes, the rate of cholesterol esterification has been proposed to determine the levels of free cholesterol available for biliary secretion, and could, if so, be coupled to the pathogenesis of gallstone disease (5, 7). However, neither the activity nor the mRNA levels of ACAT2 differed between GS and GSF (Figure 3E).

Expression of nuclear receptors and transcription factors regulating hepatic lipid metabolism

The hepatic mRNA levels of the nuclear receptor HNF4A was 43% higher in the GS (P < 0.05; Figure 2A), and a positive correlation of HNF4A mRNA was found with CYP7A1 mRNA (r=0.40, P < 0.05) and CYP27 mRNA (r=0.34, P < 0.05), but no correlation was seen with CYP8B1 mRNA. A strong positive correlation was also observed between HNF4A and ABCG5 (r=0.73, P < 0.05), between HNF4A and ABCG8 (r=0.74, P < 0.05) as well as between HNF4A and SRBI (r=0.64, P < 0.05). No differences in FXR, SHP, PGC1α, HNF1A, LXRβ, SREBP1c, and SREBP2 mRNA levels were found between the two groups (Figure 2E).
DISCUSSION

Most of the previous studies intended to better understand the molecular defects leading to gallstone disease have been carried out in animal models, and their relevance to the human condition needs confirmation. In this investigation carried out in non-obese, normolipidemic Chinese gallstone patients, a significantly higher molar percentage of biliary cholesterol occurred without simultaneous changes in the molar percentage of bile acids or phospholipids. This change results in an increased CSI, a known prerequisite for gallstone formation (3, 17). The increased mRNA expression of hepatic \( ABCG5 \) and \( ABCG8 \) in GS suggests one possible mechanism for the increase in CSI. Moreover, the observed correlation between the expression levels of these two transporters and the molar percentage of biliary cholesterol supports the likelihood of a link between the expression levels of \( ABCG5 \) and \( ABCG8 \) and biliary cholesterol secretion in human beings. Unfortunately, we were not able to measure the protein levels of \( ABCG5 \) and \( ABCG8 \). In our GS patients, \( ABCG5 \) and \( ABCG8 \) also correlated with the \( LXR\alpha \) expression, and a pronounced increase (102%) in the mRNA of \( LXR\alpha \) was seen in GS individuals consistent with the possibility that in human beings, as in mice, both \( ABCG5 \) and \( ABCG8 \) are transcriptionally regulated by \( LXR\alpha \). Therefore, our data suggest that this nuclear receptor may have played a role in the pathogenesis of gallstone disease in our Chinese patients, although no correlations were found between two other LXR regulated genes (\( ABCA1 \) and SREBP1c) and \( LXR\alpha \).

In the human \( ABCG5/ABCG8 \) transgenic mouse model, a correlation between the hepatic mRNA levels of these transmembrane transporters with biliary cholesterol was reported (22). Evidence for the correlation between \( ABCG5/ABCG8 \) expression and biliary cholesterol secretion in humans has also been found in patients with sitosterolemia – a disease caused by mutations in
either ABCG5 or ABCG8 (23) and in whom biliary cholesterol secretion is markedly diminished (24). It has been proposed that pathways independent of ABCG5 and ABCG8 also exist and contribute to cholesterol secretion into bile (21). Our findings are in line with recent studies in inbred mice challenged with a lithogenic diet (25, 26). In this case, the expression of Abcg5 and Abcg8 was increased, the level of biliary cholesterol was higher, and gallstone formation occurred. In mice, Abcg5 and Abcg8 colocalized with the murine Lith 9 gene locus. Furthermore, the murine Abcg5 and Abcg8 expression is induced by LXRα (10, 11), and this gene co-localizes with murine Lith 1 (27). Treatment of gallstone-resistant AKR mice with synthetic LXRα agonists induced gallstone formation, apparently due to an increased expression of ABCG5 and ABCG8, which in turn enhanced biliary cholesterol secretion (28). However, it should be recognized that the bulk of data obtained on the transcriptional regulation by LXR has been generated in rodent models or cell systems, either by depletion of the LXR gene or by pharmacological treatment with LXR agonists. In this case, some discrepancies between the observations in human liver and the observations from other experimental models are not unexpected. Limitations in sample size and in the opportunity for experimental manipulation of human subjects may also play a role in interpretation although overall, the similarities identified between studies in mice and human beings are encouraging.

In gallstone susceptible C57L mice, synthetic FXR ligands are reported to prevent gallstone formation by inducing expression of ABCB11 and ABCB4 (29). However, our gallstone patients did not show any differences in FXR, ABCB11, and ABCB4 expression, data which are in line with our findings on concentrations of biliary bile acids and phospholipids and also consistent with the unchanged CYP7A1 expression. These observations do not conflict with the proposed role for FXR in gallstone disease in that they do not exclude the possibility of FXR as a
successful therapeutic target in humans aimed at reduction of the supersaturation of cholesterol in bile.

The increased expression of the HDL receptor SRBI observed in our GS patients suggests that enhanced uptake of HDL cholesterol may have contributed to the increase of biliary cholesterol. This finding is consistent with our previous observations, where we found free cholesterol in HDL to be in rapid equilibrium with biliary cholesterol (30). Furthermore, in patients with a bile fistula, cholesterol from the HDL-particles was more rapidly incorporated into biliary cholesterol than cholesterol from LDL particles (31). In complete agreement with the present report is that Srb1 expression regulates biliary cholesterol secretion in mice, but not the secretion of phospholipids or bile acids (32, 33).

We did not observe any differences in plasma HDL cholesterol between GS and GSF, an observation consistent with another large study on Chinese gallstone patients (34). The increased SRBI protein expression observed in GS was not paralleled by a decrease in HDL cholesterol. This would not be an expected finding if the major determinant of plasma HDL cholesterol levels is the hepatic expression of ABCA1, as was indicated by the studies in ABCA1 liver-specific conditional knockout mice (35).

The excess in biliary cholesterol could also have originated from de novo synthesis of cholesterol in liver but neither the HMGCR expression nor the hepatic levels of lathosterol showed any differences between GS and GSF, a finding consistent with observations by others (7, 36). Nevertheless, cholesterol de novo synthesis cannot be completely excluded, since in patients with a bile fistula, a condition which greatly induces bile acid synthesis, it was found that about 30% of the biliary cholesterol represents newly synthesized cholesterol (31, 37).
Another mechanism that might contribute to an increase in biliary cholesterol is through a decrease in cholesterol esterification, leading to higher levels of free cholesterol available for secretion into the bile. However, our data do not support this hypothesis as neither differences in hepatic ACAT2 activity between GS and GSF nor differences in free cholesterol concentrations in whole liver homogenates from GS and GSF was observed. Furthermore, previous observations concluded that cholesterol levels did not differ within the microsomal membrane in patients with and without gallstones (7).

Conversion of cholesterol into bile acids is one key mechanism for disposal of excessive cholesterol from the body. Increased bile acid synthesis, measured as a rise in plasma 7α-hydroxy-4-cholesten-3-one, was observed in Chilean gallstone patients (38). It was suggested that this change could be secondary to increased intestinal losses of bile acids. The Chilean gallstone patients also had increased levels of triglycerides in plasma and liver, together with increased hepatic activity of the microsomal triglyceride transfer protein (MTTP). In contrast, we did not find differences between our groups in CYP7A1 and MTTP expression or in plasma and hepatic triglyceride levels. This discrepancy may be an indication that gallstone disease is not due to a single metabolic defect. The types of gallstone free patients selected for the reference-group (gallstone free controls) likely affect the outcome of all studies on gallstone disease since for ethical reasons, an ideal reference-group of completely healthy subjects cannot be studied. In our study and in the one on Chilean patients, the reference-groups are quite different. In ours, patients with gallbladder polyps were included among the gallstone free controls. The increased levels of DCA in the bile of these patients might be related to their disease, since an increase in DCA has previously been described to be associated with an enhanced induction of gallbladder polyps in hamsters (39). However in Asian subjects, a higher
level of DCA in healthy liver transplant donors has also been reported, suggesting that differences in biliary bile acid composition may exist between control groups of different ethnicity (40). In the Chilean study (38), the majority of the patients enrolled in the reference-group underwent surgical procedures because of gastrointestinal cancer. All the preoperative procedures, including the particular liquid or semi-liquid diets and the “intestinal preparation” (i.e. eventual laxative and prophylactic antibiotic therapy) given to those patients prior to the surgical procedures could have influenced the outcome in unknown ways. Another limitation of all the mechanistic studies performed in humans is represented by a relative small sample size. Thus in our study as in others, a risk that some differences may be missed cannot be excluded. Finally, in our GS, we found an increased expression of $HNF4A$, which is a nuclear receptor that regulates the tissue specific expression of many genes in the liver and other organs originating from the endoderm (41). In addition to the correlation between the expression of $HNF4A$ and $SRBI$ as was previously reported (42), we found a correlation between the expression of $HNF4A$ and of $ABCG5$ and $ABCG8$. Recently, a regulatory element for $HNF4A$ was identified in the human $ABCG5/ABCG8$ promoter region which could strongly regulate the expression of these two genes (43). A correlation between $HNF4A$ and $PGC-1\alpha$ and a decrease in the expression of this latter co-factor was described in a small number of Italian gallstone patients as compared with patients with gastrointestinal cancer or with liver donors (44). We could not confirm these findings, but the ethnicity of our patients was different as was the control group of our study. Another unexpected finding in our study was the significant correlation between the expression of $HNF4A$ and the expression of $LXR\alpha$ ($r=0.43$, $p<0.05$). The possibility that altered HNF4A activity contributes to the pathogenesis of gallstone disease, as in our non-obese normolipidemic Chinese subjects, is intriguing and should be addressed in future studies.
In conclusion, in our study in normolipidemic non-obese Chinese gallstone patients, the supersaturation of the bile with cholesterol was associated with an increased expression of the canalicular transporters ABCG5, ABCG8, which was possibly induced by the increased expression of hepatic LXRα. Our data also suggest that excess biliary cholesterol in gallstone patients may be derived, at least in part, from the plasma HDL cholesterol taken up via the hepatic HDL receptor, SRBI.
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REFERENCES


FIGURE LEGEND

Figure 1

Biliary and hepatic lipid composition in gallstone patients (GS) and gallstone-free patients (GSF).

A. Biliary lipid composition of gallbladder bile (GS, n=14 and GSF, n=9). Ch, Cholesterol; PL, phospholipids; and BA, bile acids.  B. Bile acid composition of gallbladder bile (GS, n=14 and GSF, n=9). CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; and UDCA, ursodeoxycholic acid.  C. Cholesterol content in liver lipid extract (GS: n=20 and GSF: n=9).  D. Triglyceride content in liver lipid extract (GS: n=22 and GSF: n=13).  E. Lathosterol content in liver lipid extract (GS: n=22 and GSF: n=13).  Data show mean ± SEM.

Figure 2

Hepatic expression of genes involved in lipid metabolism in gallstone patients (GS) and gallstone-free patients (GSF).

A. Gene expression of ABCG5, ABCG8, SRBI, LXRα and HNF4A (GS: n=22 and GSF: n=13).  Data show mean ± SEM.  B. Correlation between hepatic ABCG5 mRNA and ABCG8 mRNA levels (n=35).  C. Correlation between hepatic LXRα and ABCG5 mRNA levels (n=35).  D. Correlation between hepatic LXRα mRNA levels and cholesterol saturation index (CSI) in gallbladder bile (n=23).  E. Relative gene expression between GS (n=22) and GSF (n=13).  The dotted line at value 1 represents the mean gene expression level in GSF, which was arbitrarily set to 1 and the black bars represent the gene expression level in GS (mean ± SEM).  For all genes, no difference was found between GS and GSF.
Figure 3

Hepatic expression of SRBI and hepatic expression and activity of ACAT2 in gallstone patients (GS) and gallstone-free patients (GSF).

A. Western Blot analysis of SRBI protein (MW: 82kDa) level normalized to β-actin (MW: 42kDa) as a loading control on 10% SDS-PAGE. ‘R’ represents a human liver membrane sample that has been used as a reference for each gel. B. SRBI protein level were 74% higher in GS than in GSF. Data show mean ± SEM of the value obtained from the quantitation of the blots showed in A. C. Correlation between hepatic SRBI protein and biliary cholesterol molar percentage in gallbladder bile (n=20). D. Correlation between hepatic SRBI protein and CSI in gallbladder bile (n=20). E. Hepatic ACAT2 microsomal activity (GS: n=19 and GSF: n=11) and hepatic ACAT2 mRNA (GS: n=21 and GSF: n=13) expression in GS and GSF. Data show mean ± SEM.
### Table 1  Age, BMI and plasma lipids of gallstone patients and gallstone free controls

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<tr>
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<th>GS(n=22)</th>
<th>GSF(n=13)</th>
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<tr>
<td>Age(years)</td>
<td>45.0±2.6</td>
<td>44.1±2.9</td>
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<tr>
<td>BMI(kg/m²)</td>
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<td>21.9±0.9</td>
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<td>CHOL mmol/L</td>
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<tr>
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<tr>
<td>APO B g/L</td>
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<td>0.74 ± 0.07</td>
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Figure 1
Figure 2

Relative mRNA expression (normalized to cyclophilin A, A.U.)

ABCB11
ABCB4
ABCA1
HMGCR
NPC1L1
CYP7A1
CYP8B1
CYP27
MTP
HNF1A
SHP
FXR
LXRβ
PGC1α
SREBP1c
SREBP2
LDLR
LRP
APOA1

GSF=1

ABCG5 mRNA

r = 0.58, P < 0.05

GSF mRNA

r = 0.43, P < 0.05

ABCG8 mRNA

r = 0.89, P < 0.01

Relative mRNA expression (normalized to cyclophilin A, A.U.)

ABCG5

GSF

GS

P < 0.01

P < 0.01

P < 0.05

P < 0.01

P < 0.05

ABCG8

GSF

GS

P < 0.01

P < 0.05

LXRα

CSI

r = 0.43, P < 0.05

LXRα

r = 0.58, P < 0.05

GSF

GS

P < 0.01

P < 0.05

HNF4A

GSF

GS

P < 0.01

P < 0.05

GSF=1

GSF

GS

P < 0.01

P < 0.05
Figure 3