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ORIGINAL ARTICLE

# Association of *FXR* gene variants with cholelithiasis

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**Summary**
*Background and aim:* Impairment of bile acid homeostasis is the most important risk factor of gallstone disease. Thereby the bile acid sensor farnesoid X receptor (FXR) plays a pivotal role in hepatic and intestinal bile acid metabolism. In this explorative study, the *FXR* gene was investigated to identify gene variants, associated with gallstone formation in a Caucasian population.
*Methods:* Sequencing of the *FXR* gene was conducted in a randomly selected cohort of gallstone carriers ($n = 30$) and control subjects ($n = 16$) from Stuttgart, Germany. Genomic DNA was obtained from blood leukocytes. Genotype frequencies were established in the total cohort (controls: $n = 133$, gallstone carriers: $n = 74$). For expression analysis, total RNA and protein were isolated from ileal biopsies.
*Results:* The sequencing showed the sole appearance of 10 SNPs in gallstone carriers. Further genotype analysis revealed significant gender- and weight-dependent frequency differences of 3 SNPs between gallstone carriers and controls in males (rs35724: OR = 4.73, $P = 0.022$) and normal weight subjects (rs11110385: OR = 3.67, $P = 0.027$; rs11110386: OR = 3.67, $P = 0.027$)

*Abbreviations:* ABCB4, ATP-binding cassette, sub-family B, member 4; ABCB11, ATP-binding cassette sub-family B member 11; ABCG5/G8, ATP-binding cassette proteins G5/G8; ADRB3, β3-adrenergic receptor; ASBT, apical sodium bile acid transporter; BA, bile acid; BMI, body mass index; bp, base pair; CI, confidence interval; CYP7A1, cholesterol 7-alpha-hydroxylase; CYP7B1, 25-hydroxycholesterol 7-alpha-hydroxylase; FGF, fibroblast growth factor; FXR, farnesoid X receptor; GWAS, genome-wide association studies; HWE, Hardy-Weinberg equilibrium; ILBP, ileal lipid binding protein; LD, linkage disequilibrium; LRH1, liver receptor homologue 1; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; NTCP, Na+-taurocholate cotransporting polypeptide; OR, odds-ratio; Ostα/β, organic solute transporters α and β; RT-qPCR, Real-Time Quantitative PCR; SEM, standard error of the mean; SHP, short heterodimer partner; SNP, single nucleotide polymorphism.

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applying the 11 + 12 < > 22 allele model. Furthermore, rs11110385 carriers showed a significantly decreased FXR protein expression (11 + 12 < > 22: $P = 0.003$). Significant mRNA expression differences between lean rs11110385 carriers and non-carriers were observed in FXR target genes (decrease: ILBP: $P = 0.042$, OSTalpha: $P = 0.071$, FGF19: $P = 0.011$. Increase: LRH1: $P = 0.044$).
*Conclusions:* Three FXR gene variants (rs35724, rs11110385, rs11110386) were identified as potential susceptibility factors for cholelithiasis in a German cohort in gender- and weight-dependent manners. Thereby the tag SNP rs11110385 seemed to influence the activation of the *FXR* gene.


## Introduction

In the last decade, family and twin studies in humans as well as inbred mice have highlighted the importance of genetic risk factors, which determine the individual risk of developing cholesterol gallstones, interacting with common risk factors like gender, obesity, age and life style [1—3].

Several gene loci with linkage to cholelithiasis were described. Upon prior studies on ABCG5/G8 in mice [4] this cholesterol transporter was identified as risk gene for cholelithiasis in humans [5,6]. The association of the D19H variant of ABCG8 with gallstones was confirmed in various populations and ethnicities [7,8]. Probably D19H is associated with increased cholesterol transport into bile [8] but also from intestinal epithelium to the lumen, explaining diminished cholesterol absorption [9]. The obesity linked [10] Arg64 variant in the *ADRB3* gene was also identified to be associated with gallstone formation in Caucasians, probably affecting gallbladder motility [11]. Furthermore, genotyping analysis revealed a significant association of one ASBT gene variant and cholelithiasis in various cohorts [12,13].

These genetic approaches confirmed that in gallstone pathogenesis changes in cholesterol transport and a disrupted BA homeostasis are key determinants [14—16]. Lithogenesis in general is associated with:

- low cholesterol absorption [9,17];
- a trend towards increased cholesterol synthesis [9,17];
- diminished BA pools and increased BA turnover [18], probably related to a diminished expression of ileal BA transporters [19,20].

Notably, these parameters are also related to body weight and differ significantly between lean and overweight patients with or without cholelithiasis [19,21].

To control these metabolic pathways various nuclear receptors exert their activities to maintain BA homeostasis [22—24]. Among them, FXR plays a pivotal role as prominent biosensor of endogenous BAs in the regulation of ileal reabsorption as well as in the hepatic synthesis of BAs [22,23]. FXR is highly expressed in the gut and the liver, protecting cells by sensing intracellular BA levels and regulating BA flow in both tissues [25]. This transcription factor indirectly reduces the gene activation of ileal ASBT [26] and directly induces intracellular trafficking of BAs through ileocytes via the cytosolic ILBP [27] as well as BA export into the portal blood by the basolateral Ost$\alpha$/$\beta$ [28]. Hepatic FXR prevents the harmful consequences of pathological BA overload in the liver, enhancing bile flow and suppressing the BA influx to hepatocytes as well as rate limiting enzymes of BA synthesis CYP7A1 and CYP7B1 [23,29]. Ileal FXR functions as activator of the endocrine hormone FGF15 in mice, orthologous to human FGF19 [30,31], which suppresses BA synthesis (CYP7A1) in hepatocytes [31].

There is strong evidence for a link between FXR and cholelithiasis in animals. The *FXR* gene was identified as a possible *lith* gene contributing to cholesterol gallstone formation [3]. Activated FXR triggers an increased biliary bile salt secretion and phospholipid concentration, subsequently preventing gallstone development in mice [32]. Studies about the role of FXR in human cholesterol gallstone formation are limited. FXR (gene name: *NR1H4*) was described as a susceptibility factor for cholesterol gallstones in Mexican males [33]. Previously we found that Caucasian female lean gallstone carriers have a decreased ileal expression of FXR and its target genes *ASBT*, *ILBP*, *Ost*$\alpha$/$\beta$ [19,20].

In the current study, we investigated an association between FXR polymorphisms and gallstone development, screening the *FXR* gene for SNP variants by systematic sequencing and genotyping analysis.

## Materials and methods

### Study populations and clinical characteristics

For genetic analyses the study population from Stuttgart/Germany comprises 207 individuals (133 healthy persons, 74 gallstone carriers). Biopsies and blood samples were taken from individuals undergoing routine colonoscopy for various clinical indications. The presence or absence of gallstones was determined by abdominal ultrasound. Subjects included in this study had:

- normal serum lipid values and no history of taking lipid-lowering drugs or drugs interfering with bile acid uptake;
- no known medical conditions affecting lipid metabolism;
- normal liver function and no signs of hemolysis or other conditions associated with pigment stones;
- no intestinal surgery;