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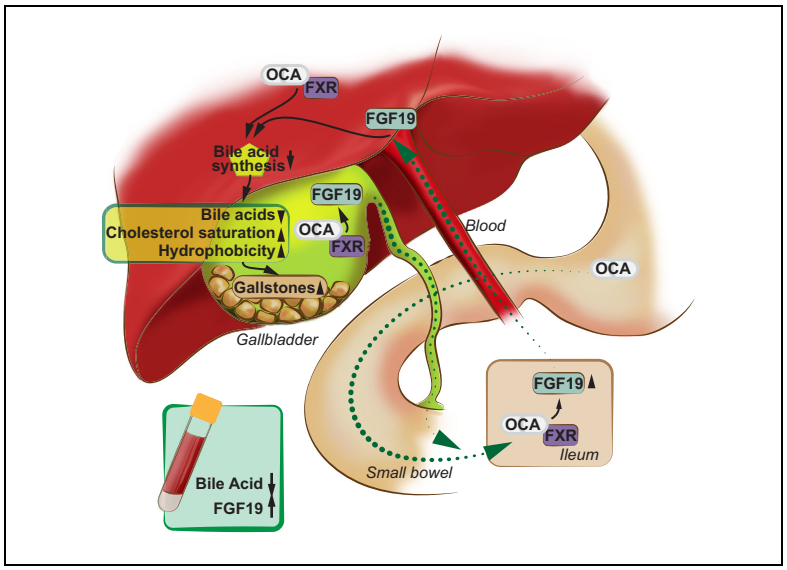
Obeticholic acid may increase the risk of gallstone formation in susceptible patients

Graphical abstract



Highlights

- In humans, FXR activation with obeticholic acid decreases gallbladder bile acids.
- FXR activation with obeticholic acid increases the biliary cholesterol saturation index.
- It also increases the bile acid hydrophobicity index and formation of cholangiocellular FGF19.
- All of these effects are potential risk factors for gallstone formation.

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Lay summary

Obeticholic acid increased human gallbladder cholesterol saturation and bile acid hydrophobicity, both decreasing cholesterol solubility in bile. Together with increased hepatobiliary levels of fibroblast growth factor 19, our findings suggest that pharmacological activation of the farnesoid X receptor increases the risk of gallstone formation.

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Obeticholic acid may increase the risk of gallstone formation in susceptible patients

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Background & Aims: The nuclear farnesoid X receptor (FXR) agonist obeticholic acid (OCA) has been developed for the treatment of liver diseases. We aimed to determine whether OCA treatment increases the risk of gallstone formation.

Methods: Twenty patients awaiting laparoscopic cholecystectomy were randomized to treatment with OCA (25 mg/day) or placebo for 3 weeks until the day before surgery. Serum bile acids (BAs), the BA synthesis marker C4 (7α-hydroxy-4-cholesten-3-one), and fibroblast growth factor 19 (FGF19) were measured before and after treatment. During surgery, biopsies from the liver and the whole bile-filled gallbladder were collected for analyses of gene expression, biliary lipids and FGF19.

Results: In serum, OCA increased FGF19 (from 95.0 ± 8.5 to 234.4 ± 35.6 ng/L) and decreased C4 (from 31.4 ± 22.8 to 2.8 ± 4.0 nmol/L) and endogenous BAs (from 1,312.2 ± 236.2 to 517.7 ± 178.9 nmol/L; all p <0.05). At surgery, BAs in gallbladder bile were lower in patients that received OCA than in controls (OCA, 77.9 ± 53.6 mmol/L; placebo, 196.4 ± 99.3 mmol/L; p <0.01), resulting in a higher cholesterol saturation index (OCA, 2.8 ± 1.1; placebo, 1.8 ± 0.8; p <0.05). In addition, hydrophobic OCA conjugates accounted for 13.6 ± 5.0% of gallbladder BAs after OCA treatment, resulting in a higher hydrophobicity index (OCA, 0.43 ± 0.09; placebo, 0.34 ± 0.07, p <0.05). Gallbladder FGF19 levels were 3-fold higher in OCA patients than in controls (OCA, 40.3 ± 16.5 ng/L; placebo, 13.5 ± 13.1 ng/ml; p <0.005). Gene expression analysis indicated that FGF19 mainly originated from the gallbladder epithelium.

Conclusions: Our results show for the first time an enrichment of FGF19 in human bile after OCA treatment. In accordance with its murine homolog FGF15, FGF19 might trigger relaxation and filling of the gallbladder which, in combination with increased cholesterol saturation and BA hydrophobicity, would enhance the risk of gallstone development.

Lay summary: Obeticholic acid increased human gallbladder cholesterol saturation and bile acid hydrophobicity, both decreasing cholesterol solubility in bile. Together with increased hepatobiliary levels of fibroblast growth factor 19, our findings suggest that pharmacological activation of the farnesoid X receptor increases the risk of gallstone formation.

Clinical trial number: NCT01625026.

Introduction

Bile acids (BAs) are amphipathic molecules that are synthesized from cholesterol in the liver. Once synthesized, they are conjugated with glycine or taurine and then excreted with bile into the small bowel from where about 95% are reabsorbed in the terminal ileum via the enterohepatic circulation. In addition to their detergent properties that aid lipid digestion, BAs serve as signaling molecules by activating various nuclear and membrane-bound receptors, in particular the nuclear farnesoid X receptor (FXR), which regulates BA, glucose and lipid metabolism.[1,2] BA homeostasis is maintained through negative feedback activation of FXR both within the liver (via SHP-LRH-1/HNF-4α) and from the small intestine (via FGF19-FGFR4/ß-klotho) by decreasing the expression of cholesterol 7α-hydroxylase (CYP7A1), the key enzyme in BA synthesis.[3,4]

The potent FXR agonist obeticholic acid (OCA, 6α-ethyl-chenodeoxycholic acid) has been developed for the treatment of various cholestatic and metabolic liver diseases such as primary biliary cholangitis (PBC) and non-alcoholic steatohepatitis (NASH).[5,6] Currently, OCA is provisionally approved as a second-line treatment option in PBC. It is important to note that the downstream effects of FXR activation in humans are largely unknown. Almost all studies exploring these mechanisms have been conducted in murine models; however, because of substantial differences in BA metabolism and BA profiles between mice and humans, it is difficult to translate findings from these models into humans.[1,2]

Herein, we aimed to decipher the downstream actions of FXR activation on human gallbladder and liver by studying the effects of OCA in a randomized double-blind placebo-controlled trial. We hypothesized that decreased synthesis and

Keywords: Obeticholic acid; Farnesoid X receptor; FGF19; Cholesterol saturation index; Hydrophobicity index; Gallstones.
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excretion of BAs from the liver would affect gallbladder lipid composition resulting in an increased risk of gallstone formation. Of note, with respect to hepatobiliary events, the recent interim analysis of the ongoing REGENERATE trial in patients with NASH reported that more patients (3%) on OCA 25 mg experienced gallstones or cholecystitis than those who received placebo (<1%) or OCA 10 mg (1%).[7]

Materials and methods

Study population and protocol

In a double-blinded placebo-controlled randomized trial (Obeticholic Acid in Gallstone Surgery [OCAGS], Clinical Trials NCT01625026), 20 patients awaiting laparoscopic gallstone surgery with symptomatic gallstone disease but without significant comorbidity or medication known to affect glucose or lipid metabolism were randomized to OCA (25 mg/day, which is the same dose as in the OCA NASH trial[6]) or matching placebo for 3 weeks until the day before surgery. Inclusion and exclusion criteria, as well as the clinical research file of our patients with symptomatic gallstone disease, were the same as in our previous study where we compared the molecular effects of rifampicin and ursodeoxycholic acid.[8] Thus, exclusion criteria included previous or ongoing liver, kidney, intestinal or metabolic diseases, and the use of medications known to affect liver function and metabolism, *e.g.* lipid- or glucose-lowering drugs and P450 enzyme inducers. Importantly, patients were not prescribed any low-calorie regimen before surgery. Informed consent was obtained from all patients.

Blood samples for biochemistry were collected from patients in the fasting state on the days immediately before study start and surgery. During surgery, approximately 1 cm long harmonic-knife biopsies from the liver and the whole bile-filled gallbladder (in this order) were collected. Bile was aspirated with a syringe after puncturing the gallbladder; the gallbladder was thereafter cut into pieces for tissue samples. Due to suspicion of gallbladder polyps, 1 gallbladder from each group was sent away for pathological evaluation and thus not available for molecular biology analyses. Tissues and bile aliquots were frozen immediately in liquid nitrogen. All samples were kept frozen at −80 °C until analysis.

The study was approved by the Regional Ethical Committee in Gothenburg (Dnr 199/11; 08JUN2011) and the Swedish Medical Products Agency (EudraCT 2011-0008-13-37; 07MAY2012). All authors had access to the study data and have reviewed and approved the final manuscript.

Analysis of bile acids, C4 and FGF19

BAs and C4 (7α-hydroxy-4-cholesten-3-one) were analyzed by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) as previously described.[9] FGF19 was measured by ELISA according to the manufacturer's instructions (Quantikine ELISA kit, R&D Systems).

Cholesterol saturation index and bile acid hydrophobicity index

The cholesterol saturation index was calculated according to Carey's critical tables.[10] The hydrophobicity index was calculated according to Heuman.[11]

RNA isolation and qPCR

RNA from human liver and gallbladder tissue was isolated following the RNeasy protocol (Qiagen, RNeasy Mini kit) including DNAse I digest on column. RNA concentrations were measured by Nanodrop and 1 μg of RNA was subjected to cDNA synthesis (Superscript III, Invitrogen). Quantitative PCR (qPCR) was performed on a Light Cycler (Roche) with Luna Universal qPCR Master Mix (NEB).

All qPCR data were normalized to 36b4. Because RNA was isolated from full thickness gallbladder tissue and the proportion of epithelial versus non-epithelial tissue varied among different samples, gallbladder values were normalized to CK19 using the following formula:

$K_{CK19}=\Delta_{CK19} - \mathrm{Min}(\Delta_{CK19})$

$FGF19_{norm} = 2\text{^}-(\Delta_{FGF19} - K_{CK19})$

The $FGF19_{norm}$ gallbladder values (with and without OCA) were compared to the $2\text{^}-(\Delta_{FGF19})$ liver values (with and without OCA).

Statistical analysis

All data are reported as mean ± SD, unless otherwise indicated. Serum BAs and BAs, cholesterol and phospholipids in gallbladder bile were normally distributed both in the OCA and placebo groups. *P* values were calculated with Student's *t* test unless otherwise indicated. A p value <0.05 was considered significant.

Results

Patient demographics and clinical outcome

The groups were well matched with no significant differences in sex, age, body mass index, absence of insulin resistance as estimated by homeostatic assessment of insulin resistance (HOMA-IR), and baseline laboratory findings (Table 1). All patients finished the study per protocol. A total of 7 adverse events were reported: 1 common cold and 1 case of pruritus in both the OCA and placebo groups, 1 case of heartburn in the OCA group, 1 headache and 1 sleep disturbance in the placebo group. There were no serious adverse events. In the OCA group, there were minor yet significant increases in low-density lipoprotein cholesterol and alkaline phosphatase and decreases in gamma-glutamyltransferase after treatment (Table 1), confirming observations in the NASH trial with OCA.[6]

OCA decreases bile acid synthesis

OCA treatment promoted dramatic decreases in concentrations of the serum BA synthesis marker 7α-hydroxy-4-cholesten-3-one (C4) and endogenous BAs (Table 1). OCA constituted 44.3% of the total serum BAs (Table 1) and was present almost entirely as either glycine (54.4%) or taurine (44.9%) conjugates. OCA conjugates, which are substrates of the ileal bile acid transporter (IBAT [also known as SLC10A2]),[12] are taken up in the ileum and also by the liver. The decreased BA synthesis was therefore likely a result of negative feedback promoted by OCA and its conjugates both indirectly by activation of intestinal FXR and increased circulating FGF19 (Table 1) and directly by activation of FXR in the hepatocytes.[3]

OCA increases the cholesterol saturation index in gallbladder bile

The concentration of total lipids (BAs, cholesterol, and phospholipids) in the gallbladder was significantly lower in OCA-versus placebo-treated patients (OCA, 102.1 ± 55.3 mmol/L; placebo, 228.0 ± 95.5 mmol/L; p <0.01; Fig. 1). OCA treatment increased hepatic gene expression of the bile salt export pump (BSEP [or ABCB11]) (Fig. 2), a mechanism that was found to protect

Table 1. Demographics, serum liver enzymes and parameters of bile acid metabolism.

		Placebo		OCA[#]
Age (years)		50.8 ± 13.3		48.8 ± 8.9
Sex (women/men)		8/2		8/2
	Day 1	**Day 21**	**Day 1**	**Day 21**
BMI and insulin sensitivity				
BMI (kg/m^2)	27.5 ± 9.0	27.6 ± 8.9	28.8 ± 6.0	28.7 ± 6.0
Glucose (mmol/L)	5.3 ± 0.7	5.4 ± 0.5	5.7 ± 1.0	5.9 ± 1.4
HOMA-IR	2.9 ± 2.6	3.0 ± 2.8	1.6 ± 1.0	1.7 ± 1.0
Serum liver enzymes				
ALT (U/L)	22.7 ± 11.7	45.9 ± 33.4	25.6 ± 4.9	27.1 ± 3.5
AST (U/L)	21.8 ± 5.7	35.3 ± 19.1	20.6 ± 3.9	23.7 ± 2.9
ALP (U/L)	64.0 ± 32.5	59.9 ± 32.5	78.0 ± 40.4	85.5 ± 40.6*
GGT (U/L)	23.9 ± 10.9	35.3 ± 19.1	39.4 ± 40.8	29.5 ± 31.3*
Serum lipids				
Total cholesterol (mmol/L)	4.8 ± 2.2	5.4 ± 2.6	5.1 ± 2.4	5.2 ± 2.6
LDL-cholesterol (mmol/L)	3.1 ± 1.6	3.3 ± 1.7	2.1 ± 1.2	2.3 ± 1.2*
HDL-cholesterol (mmol/L)	1.5 ± 0.7	1.7 ± 0.9	1.7 ± 0.8	1.5 ± 0.8
Triglycerides (mmol/L)	1.6 ± 1.4	3.4 ± 2.1	0.8 ± 0.4	0.6 ± 0.3
FGF19/bile acid synthesis				
FGF19 (ng/L)	99.2 ± 23.9	126.1 ± 24.5	95.0 ± 8.5	234.4 ± 35.6*
C4 (nmol/L)	29.1 ± 20.1	25.1 ± 19.1	31.4 ± 22.8	2.8 ± 4.0*
Serum bile acids				
Total bile acids (nmol/L)	1,393.8 ± 324.1	1,181.3 ± 125.9	1,312.2 ± 236.2	929.6 ± 260.1*
Endogenous bile acids (nmol/L)	1,393.8 ± 324.1	1,181.3 ± 125.9	1,312.2 ± 236.2	517.7 ± 178.9*
Obeticholic acid (nmol/L)	n.d.	n.d.	n.d.	411.9 ± 103.3*

Data are mean ± SD unless otherwise indicated.
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; C4, 7α-hydroxy-4-cholesten-3-one; FGF19, fibroblast growth factor 19; GGT, gamma-glutamyltransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OCA, obeticholic acid.
[#] No difference in any baseline data between placebo and OCA.
* p <0.05 *vs.* day 1, paired t test.

against gallstone development during FXR activation in mice.[13] However, this response did not compensate for the significantly reduced BA synthesis after OCA treatment in our human study (Table 1), as the difference in total gallbladder lipids was explained by a lower concentration of gallbladder BAs in OCA-versus placebo-treated patients (OCA, 77.9 ± 53.6 mmol/L; placebo, 196.4 ± 99.3 mmol/L; p <0.01; Fig. 1). There were no significant differences in gallbladder cholesterol or phospholipid concentrations between the groups (Fig. 1) and, in agreement, hepatic gene expression levels of their transporters ABCG5/8 and MDR3 (ABCB4) did not differ between OCA- and placebo-treated patients (Fig. 2).

The lower proportion of gallbladder BAs as molar percentage of total lipids in OCA- versus placebo-treated patients (OCA, 74.8%; placebo, 84.9%) led to a significantly increased cholesterol saturation index in the OCA group (OCA, 2.8 ± 1.1; placebo, 1.8 ± 0.8; p <0.05). These findings are in contrast to results

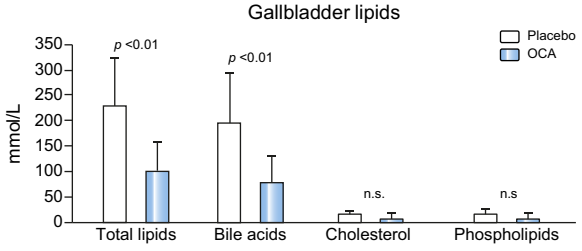

Fig. 1. Gallbladder lipid content in patients treated with placebo or OCA. The lower lipid content in OCA-treated patients is entirely caused by lower bile acid content. n = 10/group. Data are mean ± SD. p, unpaired t test. OCA, obeticholic acid.

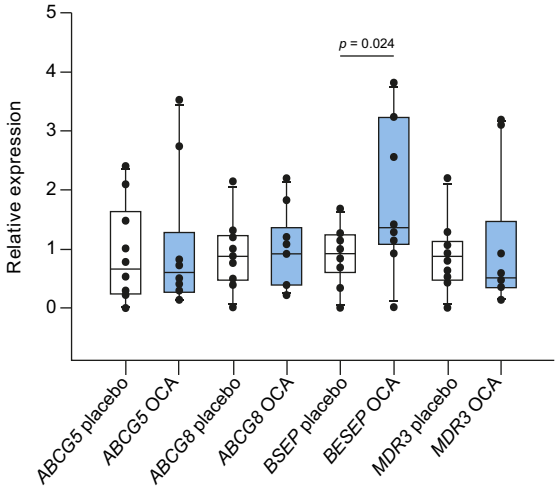

Fig. 2. Gene expression levels of hepatic canalicular transport proteins. Transport proteins for cholesterol (ABCG5, *ABCG5*), bile salts (BSEP; *ABCB11*), and phospholipids (MDR3, *ABCB4*). Significant difference between placebo and OCA-treated patients only for BSEP. n = 10/group. Data are mean and interquartile range. p, unpaired t test.

obtained in gallstone-susceptible mice where FXR activation led to increased gene expression of canalicular BA and phospholipid transporters ABCB11 and ABCB4 resulting in increased concentrations of gallbladder BAs and phospholipids, and consequently, decreased cholesterol saturation index.[14] However, the genetically susceptible mice only developed gallstones when fed a cholic acid-enriched lithogenic diet,[14] and this specific experimental model condition may, at least in part, explain the difference between the human and mice results.

OCA increases the bile acid hydrophobicity index in gallbladder bile

We next determined the hydrophobicity of gallbladder BA composition. Taurine- and glycine-conjugated OCA accounted for 13.6 ± 5.0% of gallbladder BAs in OCA-treated patients (Fig. 3A). In our reversed phase ultra-performance liquid chromatography system, OCA and its conjugates eluted close to the most hydrophobic BA lithocholic acid, indicating that they are highly hydrophobic BAs (Fig. 3B). By plotting our observed retention times against the BA hydrophobicity indices published by Heuman,[11] we calculated an individual hydrophobicity index for each BA and then a total hydrophobicity index by using the molar fractions of BAs in each sample (Fig. 3B). We showed that the BA composition was significantly more hydrophobic following OCA treatment (hydrophobicity index: OCA, 0.43 ± 0.09; placebo, 0.34 ± 0.07; p <0.05). This increase in BA hydrophobicity combined with the decreased concentration of total gallbladder BAs likely shift the gallbladder lipid composition into a physicochemical state that promotes the initiation of cholesterol crystallization.[10,11,15]

OCA increases gallbladder FGF19 which mainly originates from the gallbladder epithelium

In our study, we observed 3-fold higher concentrations of gallbladder FGF19 in the OCA- versus placebo-treated patients (OCA, 40.3 ± 16.5; placebo, 13.5 ± 13.1 ng/ml; p <0.005). In contrast to mice, which do not express FGF15 (the murine homolog of FGF19) in the hepatobiliary tract,[14,16] FGF19 has recently been shown to be expressed in the liver of humans with extrahepatic cholestasis.[16] Although RNA sequencing of gallbladder and liver tissue revealed FGF19 in both organs, FGF19 gene expression in the liver was low in both the OCA and placebo groups (Fig. 4A). By contrast, there was a robust expression and induction of FGF19 in the gallbladder of the OCA-treated group (Fig. 4A). Gene-specific qPCR analyses confirmed that *FGF19* gene expression in both the OCA and placebo groups was low in the liver and higher in the gallbladder (Fig. 4B). Furthermore, OCA treatment promoted a tendency to increase *FGF19* in the gallbladder (Fig. 4B). Given this trend and the fact that *FGF19* gene expression was low in the liver, we propose that most gallbladder FGF19 is synthesized *in loco*. Expression of the FXR target OSTα (encoded by SLC51A) was increased by OCA, confirming activation of FXR following OCA treatment (Fig. 4B).

Discussion

In our randomized double-blind placebo-controlled pharmacodynamic trial of the BA-derived FXR agonist OCA, we tested the hypothesis that decreased synthesis and excretion of BAs from the liver would affect gallbladder lipid composition resulting in an increased risk of gallstone formation. We found that OCA not only significantly increased the cholesterol saturation index but also the hydrophobicity index of the gallbladder bile acid composition, both established risk factors in gallstone pathogenesis.[10,11,17] Furthermore, we found increased FGF19 in gallbladder bile. Although the physiological function of FGF19 in human gallbladder is still undefined, the hypothesized role of FGF19 is gallbladder relaxation.[16,18] This hypothesis is based on studies in mice where intravenously injected recombinant FGF15 or FGF19 has been shown to induce gallbladder relaxation and refilling.[14] Biliary FGF19 may also modulate the secretion of mucin[18,19] which is a nucleating factor for cholesterol gallstones.[17] The formation of gallstones could thus be further facilitated by decreased gallbladder emptying resulting from an increase in biliary FGF19.

Importantly, because all our patients had established symptomatic gallstone disease, we could not study cholesterol crystallization or perform kinetic analyses of gallbladder filling/relaxation. However, these studies would have generated questionable findings as adjustments for number and size of stones within the gallbladder would have been necessary.

In conclusion, this is the first report in humans describing a significant enrichment of FGF19 in gallbladder bile upon pharmacological FXR activation with OCA. FGF19 might increase gallbladder filling and relaxation which, together with significantly increased cholesterol supersaturation and BA hydrophobicity, would further increase the risk of gallstone formation in susceptible individuals. Therefore, increased vigilance is recommended.

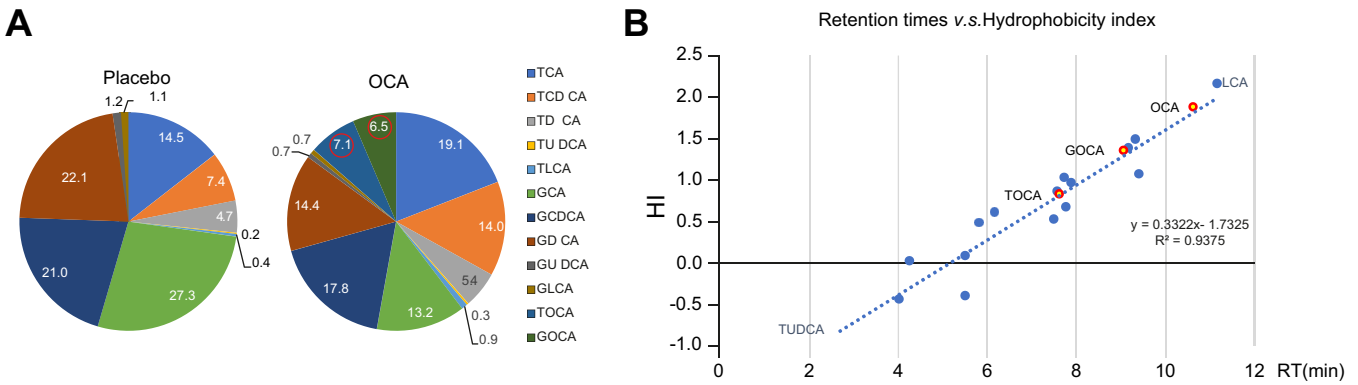

Fig. 3. Bile acid content and retention times. (A) Relative amounts (%) of bile salts in gallbladder bile of placebo- and OCA-treated patients. Glycine- and taurine-conjugated OCA (circled in red) accounted for 13.6% of the total in the OCA group. n = 10/group. (B) Retention times of bile acid standards on ultra-performance liquid chromatography-tandem mass spectrometry *vs.* published HIs[11]) spanning from TUDCA to LCA. Calculated HI for TOCA, 0.81; for GOCA, 1.34, for unconjugated OCA, 1.91. n = 10/group. GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; GOCA, glycine-conjugated OCA; GUDCA, glycoursodeoxycholic acid; HIs, hydrophobicity indices; LCA, lithocholic acid; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; TLCA, taurolithocholic acid; OCA, obeticholic acid; TOCA, taurine-conjugated OCA; TUDCA, tauroursodeoxycholic acid.

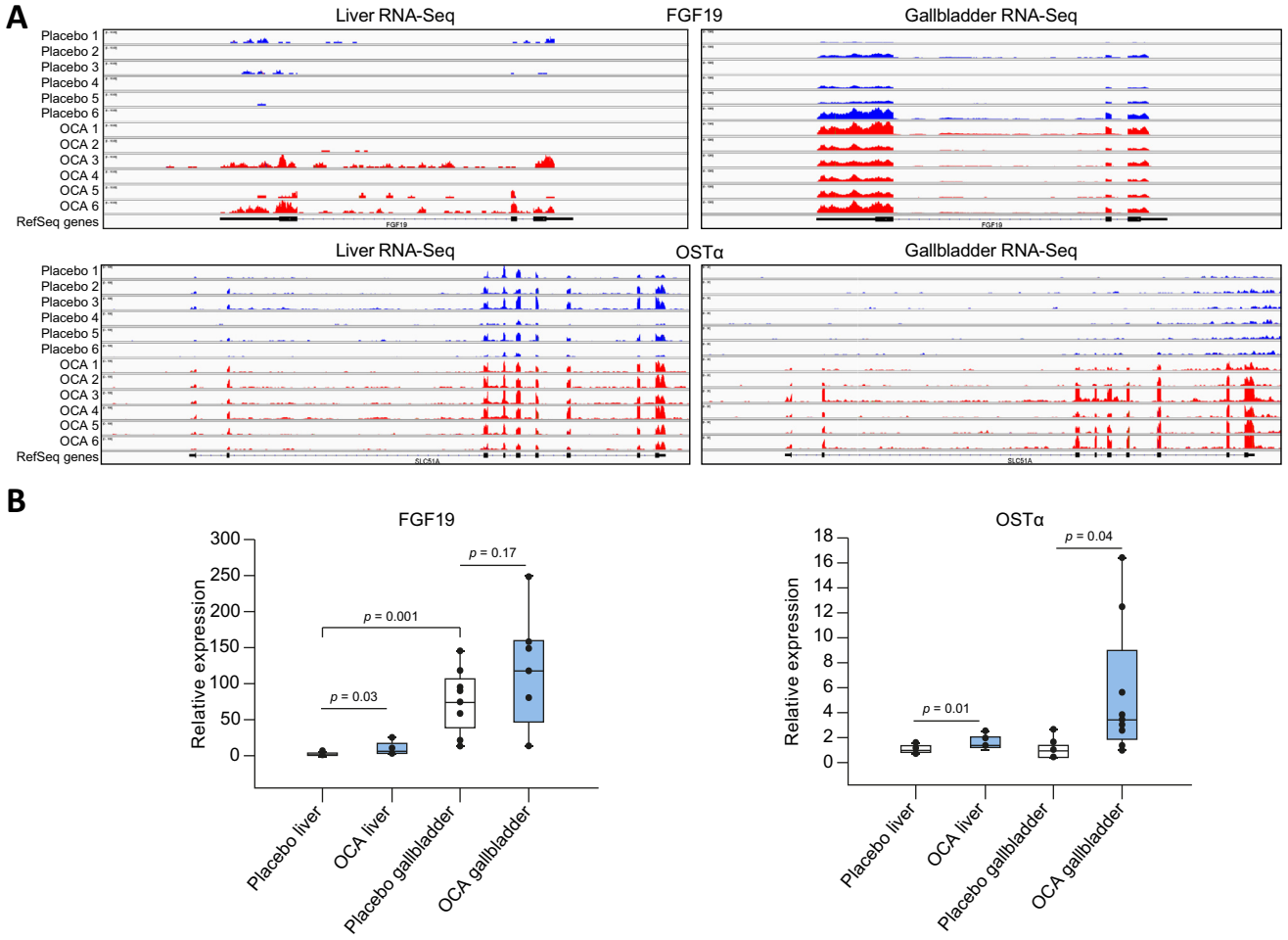

Fig. 4. Expression levels of FGF19 and OSTα. (A) Liver and gallbladder RNA-seq for FGF19 and FXR target OSTα (SLC51A). Blue lanes: patients receiving placebo; red lanes, patients receiving OCA. A number of samples from each group did not meet the required criteria for a high-quality RNA-seq run (*i.e.* RNA integrity number >6.5), and 6 RNA-seq lanes per group that are representative of samples that met the criteria are shown. (B) Liver and gallbladder qPCR of FGF19 and OSTα. Significance levels estimated by Mann-Whitney Rank Sum Test. Data are mean and interquartile range. n = 9/group (RNA of all samples were judged to be of sufficient quality for qPCR). FGF19, fibroblast growth factor 19; FXR, farnesoid X receptor; OCA, obeticholic acid; qPCR, quantitative PCR; RNA-seq, RNA sequencing.

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Conflicts of interest

HUM: Advisory boards and study drugs from Albireo and Intercept; unrestricted research grant from Intercept; MT: Speaker for BMS, Falk Foundation, Gilead and MSD; advisory boards for Albireo, Falk Pharma GmbH, Genfit, Gilead, Intercept, MSD, Novartis, Phenex and Regulus; unrestricted research grants from Albireo, Cymabay, Falk, Gilead, Intercept, MSD and Takeda; PF: Speaker for Falk Foundation; advisory boards for Dr. Falk Pharma GmbH and Intercept; unrestricted research grants from Falk Foundation and Gilead Sciences Inc; MT and PF are co-inventors of patents on the medical use of norUDCA filed by the Medical University of Graz. All other authors declare no conflict of interest.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Study concept and design: FB, LF, PF, MT, HUM. Study supervision and acquisition of material: AT and HUM. Analysis and interpretation of data: AW, KP, MS, MW, SAD, HUM. Drafting of the manuscript: SAD and HUM. Critical revision of the manuscript for important intellectual content: All authors.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2019.06.011.

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