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Short-term obeticholic acid treatment does not impact cholangiopathy in *Cyp2c70*-deficient mice with a human-like bile acid composition

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ABSTRACT

Cyp2c70$^{-/-}$ mice with a human-like bile acid (BA) composition, lacking hydrophilic muricholic acids (MCAs), have been reported to display cholangiopathy and biliary fibrosis with female preponderance that can be reversed by ursodeoxycholic acid (UDCA). Obeticholic acid (OCA), a steroidal BA-like FXR agonist, has been shown to improve liver function in patients with primary biliary cholangitis and is approved as second-line treatment for patients with an inadequate response or intolerance to UDCA. Here, we investigated the impact of OCA on BA hydrophobicity and cholangiopathy in *Cyp2c70*$^{-/-}$ mice. Male and female wild-type (WT) and *Cyp2c70*$^{-/-}$ mice were fed a chow diet with or without 10 mg/kg/day OCA for 4 weeks. OCA accounted for 1–5% of biliary BAs, with larger enrichments in *Cyp2c70*$^{-/-}$ than in WT mice. In WT mice, OCA induced a more hydrophilic, MCA-rich BA pool. In *Cyp2c70*$^{-/-}$ mice, however, BA pool became more hydrophobic with a larger proportion of chenodeoxycholic acid, attributable to a reduction of BA 12α-hydroxylation. OCA treatment reduced fecal BA excretion, indicating repression of hepatic BA synthesis in both WT and *Cyp2c70*$^{-/-}$ mice. OCA did, however, not impact on markers of liver (dys)function in plasma nor did it ameliorate cholangiopathy and fibrosis in male or female *Cyp2c70*$^{-/-}$ mice. OCA treatment also did not affect the expression of genes involved in fibrosis, inflammation and cellular senescence. In conclusion, 4 weeks of OCA treatment oppositely modulates the hydrophobicity of the BA pool in WT and *Cyp2c70*$^{-/-}$ mice, but does not improve or worsen the characteristic sex-dependent liver pathology in *Cyp2c70*$^{-/-}$ mice.

1. Introduction

Bile ducts constitute the route for transport of bile from hepatocytes to the gallbladder and small intestine. Cholangiopathies, i.e., diseases primarily affecting the bile ducts, are relatively rare diseases with various etiologies [1]. Primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) are two major cholangiopathies: PBC predominantly affects middle-aged women, while PSC can develop from childhood onwards in a male: female ratio of 2:1 [2]. PBC and PSC share some pathological features, such as inflammation and progressive destruction of bile ducts, predominantly affecting small intrahepatic bile ducts in PBC and medium-sized to large intrahepatic or extrahepatic bile ducts in PSC, development of cholestasis, liver fibrosis and eventually cirrhosis [3]. Biliary fibrosis is a key determinant of clinical prognosis in both diseases [4]. Currently, ursodeoxycholic acid (UDCA) is the standard treatment for patients with PBC, however, with a response rate of only 60% [5]. Obeticholic acid (OCA), a chemically-synthesized derivative of chenodeoxycholic acid (CDCA) [6], is a potent Farnesoid X Receptor (*FXR/NR1H4*) agonist that has been approved for PBC patients showing an inadequate response to UDCA or are intolerant to this treatment. There is no approved medical treatment for PSC patients yet.

The bile acid (BA)-activated FXR is an important regulator of BA homeostasis in the body that modulates a variety of processes, including inhibition of BA synthesis, stimulation of BA efflux from hepatocytes

Abbreviations: Cyp2c70, cytochrome P450, family 2, subfamily C, polypeptide 70; F, female; M, male; WT, wild-type; KO, knock out; BA, bile acid; FXR, farnesoid X receptor; OCA, obeticholic acid; TOCA, taurine-conjugated OCA; CA, cholic acid; TCA, taurocholic acid; CDCA, chenodeoxycholic acid; TCDCA, taurochenodeoxycholic acid; αMCA, α muricholic acid; βMCA, β muricholic acid; ωMCA, ω muricholic acid; UDCA, ursodeoxycholic acid; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; HI, hydrophobicity index; Cyp8b1, sterol 12-alpha-hydroxylase.

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and, thereby, prevention of BA accumulation within the liver [7,8]. In addition, activation of FXR by OCA has been reported to exert anti-inflammatory and anti-fibrotic effects in several animal models of liver disease [9–11]. Clinical trials have demonstrated beneficial effects of OCA treatment on plasma markers of liver function, including alkaline phosphatase (ALP) and total bilirubin, in PBC patients [12]. This has led to FDA-approval of OCA as a second-line therapy for PBC patients with an incomplete response to UDCA in 2016. OCA has also been evaluated in patients with PSC [13] and an intermediate analysis shows beneficial effects, including a reduction of serum ALP levels. Collectively, OCA appears to be a promising candidate in the treatment of cholangiopathies. However, the efficacy of OCA to reduce biliary fibrosis and to halt disease progression in PBC and PSC patients is still poorly defined.

Recently, our group has generated *Cyp2c70* knockout (*Cyp2c70*$^{-/-}$) mice, a mouse model with a relatively hydrophobic, human-like, BA pool composition that develops cholangiopathy and biliary fibrosis in a time- and gender-dependent manner [14]. CYP2C70 is the enzyme that mediates mouse/rat-specific muricholic acid (MCA) synthesis and, thereby, is responsible for the major difference in BA composition between mice and humans, that lack MCA in their BA pool. Thus, like humans, *Cyp2c70*$^{-/-}$ mice only synthesize CDCA and cholic acid (CA) as primary BAs and are devoid of MCAs [15]. Accordingly, the hydrophobicity index (HI), which defines the hydrophilic-hydrophobic balance of biliary BAs, increases from −0.3 (hydrophilic) in wild-type (WT) mice to +0.3 (hydrophobic) in *Cyp2c70*$^{-/-}$ mice, i.e., a value that is very similar to the HI in human bile [16]. Possibly, as a consequence of their relatively hydrophobic BA pool, however, ductular reactions, inflammation and portal fibrosis are manifest in livers of *Cyp2c70*$^{-/-}$ mice. Hence, *Cyp2c70*$^{-/-}$ mice may serve as a mouse model to screen the efficacy of novel therapies for cholangiopathies. Intriguingly, hepatic expression of *Cyp8b1,* encoding the enzyme responsible for BA 12α-hydroxylation and thereby for CA formation*,* is reduced more strongly in female than in male *Cyp2c70*$^{-/-}$ mice, which translates into a larger proportion of hydrophobic CDCA in the BA pool of female *Cyp2c70*$^{-/-}$ mice compared to males. Likely as a consequence hereof, features of liver disease are more pronounced in female *Cyp2c70*$^{-/-}$ mice. Treatment with the hydrophilic BA UCDA, the first-line therapy for PBC, restores elevated plasma transaminase levels as well as proliferation of cholangiocytes and fibrosis in livers of female *Cyp2c70*$^{-/-}$ mice [14]. Therefore, in the current study, we explored the impact of OCA on both mild and more advanced cholangiopathy and liver fibrosis in male and female *Cyp2c70*$^{-/-}$ mice, respectively. OCA treatment inhibited BA synthesis in WT and *Cyp2c70*$^{-/-}$ mice of both genders, but oppositely modulated BA pool hydrophobicity in WT and *Cyp2c70*$^{-/-}$ mice due to an inhibition of BA 12α-hydroxylation. However, cholangiopathy, liver fibrosis as well as indicators of inflammation and cellular senescence in *Cyp2c70*$^{-/-}$ mice remained unaffected after 4 weeks of OCA treatment.

2. Materials and methods

2.1. Animals and treatment protocols

12 weeks old male and female *Cyp2c70*$^{-/-}$ mice [14] and their WT littermates were used in this study. Mice were fed a standard chow diet (Ssniff RM maintenance diet, Bio services, Uden, the Netherlands) with or without 62.5 mg/kg obeticholic acid (OCA, Cat.# HY-12222; MedChemExpress, Monmouth Junction, NJ, USA), corresponding to an intake of ~10 mg/kg/day, for 4 weeks (n = 7–11 mice/group). Mice were housed in a temperature-controlled room (21 °C) with a 12 h-light/12 h-dark cycle and had ad libitum access to food and drinking water. Body weight and food intake were monitored weekly. At the end of the experiment, mice were sacrificed and blood and organs were collected for analysis. All animal experiments were performed in accordance with EU directive 2010/63/EU for animal experiments and protocols were approved by the Dutch Central Committee for Animal Experiments and the Animal Welfare Body of the University of Groningen. Data were reported in accordance with the ARRIVE guidelines [17].

2.2. Measurement of bile acids in plasma, bile and feces

BAs in plasma and gallbladder bile were analyzed by ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS/MS), as described in [18]. The enrichment of OCA and its taurine conjugate, as well as the ratio of 12α- to non-12α-hydroxylated BAs in both plasma and bile were determined. The HI of biliary BAs was calculated based on the Heuman index [16]. The HIs for taurine-conjugated OCA and unconjugated OCA were 1.00 and 1.24, respectively, as estimated by linear regression of the retention times of individual BA species and known Heuman's HI values. Feces samples were collected for 72 h prior to termination. 50 mg of dried and ground feces was used for BA extraction, which was incubated in 2 mL of alkaline methanol at 80 °C for 3 h, followed by a 15 mins ultrasonic batch. A mixture of samples and methanol in a ratio of 1:20 went through the solid phase extraction in Oasis HLB Cartridge (Waters, Milford, MA, USA). BAs were eluted by methanol and dried under a stream of nitrogen at 50 °C. Samples were then redissolved in 50% methanol right before the UHPLC-MS/MS measurement. A Nexera X2 Ultra High Performance Liquid Chromatography system (SHIMADZU, Kyoto, Japan), coupled to a SCIEX QTRAP 4500 MD triple quadrupole mass spectrometer (SCIEX, Framingham, MA, USA) was used. BAs were separated with a ACQUITY UPLC BEH C18 Column (1.7 μm × 2.1 × 100 mm) equipped with a ACQUITY UPLC BEH C18 VanGuard Pre-Column (1.7 μm × 2.1 × 5 mm) (Waters, Milford, MA, USA). Separation was achieved in 28 min using 10 mM ammonium acetate in 20% acetonitrile (mobile phase A) and 10 mM ammonium acetate in 80% acetonitrile (mobile phase B). Flow rate was set at 0.4 mL/min. Quantification of each BA species was calculated from a linear standard curve using an deuterium labeled internal standard.

2.3. Histology and immunohistochemical staining

Examination of liver histology was performed on 4 μm sections of formalin-fixed, paraffin-embedded tissue. Hematoxylin-eosin (H&E) staining was performed according to standard protocols. For Fast Green/Sirius Red staining, rehydrated de-paraffinized liver sections were incubated in a solution of saturated picric acid containing 0.1% Direct Red 80 (Cat.# 365,548, Sigma-Aldrich, St. Louis, MO) and 0.1% Fast Green FCF (Cat.# F7252, Sigma-Aldrich, St. Louis, MO). Immunohistochemical staining for CK19 was performed using a 1:500 diluted primary anti-CK19 antibody (Ab52625, Abcam, Cambridge, UK) and a secondary Goat anti-rabbit/HRP antibody (P0448, Dako, Glostrup, Denmark). Immune complexes were visualized using diaminobenzidine (SK-4100, Vector Laboratories, Burlingame, CA, USA). Images were automatically scanned by a Hamamatsu NanoZoomer (Hamamatsu Photonics, Almere, The Netherlands). Quantification of positive Sirius Red areas and CK19 areas was performed using Image J (v1.53a, National Institutes of Health, Bethesda, MD).

2.4. Hepatic hydroxyproline measurement

Liver homogenates (15% *w/v* in demi water) were sonicated and hydrolyzed in 1 mL 6 N HCl at 110 °C for 20 h in sofirell tubes. L-Phenyl-d5-alanine (Cambridge Isotope Laboratories, Tewksbury, MA, USA) was used as an internal standard to correct for any potential evaporation during heating. After cooling down to room temperature, the hydrolysate was neutralized by addition of an equal volume of 6 N NaOH. This solution was transferred to a VWR centrifugal filter (VWR International, Pennsylvania, USA) and centrifuged at 2000 xg, at 4 °C for 10 min. Then, 10 μL of the sample mixed with 10 μL internal standard mix (100 μM isotope labeled amino acids) and 100 μL methanol was taken for hydrophilic interaction liquid chromatography-mass spectrometry (HILIC/

MS) analysis. Chromatographic separation was achieved using an ACQUITY UPLC BEH Amide Column (1.7 μm, 2.1 mm × 100 mm) (Waters, Milford, Massachusetts, USA) and a Shimadzu Nexera UHPLC system (Kioto, Japan). Mobile phase A consisted of 0.1% formic acid in 10 mM ammoniumformate dissolved in 100% MilliQ-water while mobile phase B consisted of 0.1% formic acid in 10 mM ammoniumformate dissolved in 95% Acetonitril/MilliQ-water. The flow rate was set at 0.4 mL/min. Sciex Analyst®MD 1.6.2 and Sciex Multiquant®MD 3.0.3 software (Framingham, MA, USA) were used for data processing. The concentration of hepatic hydroxyproline was calculated from a linear standard curve of trans-4-hydroxy-L-proline-d4 (Toronto Research Chemicals, North York, Canada) and expressed as μg/g wet tissue.

2.5. RNA extraction and gene expression

Total RNA was extracted from liver and ileum using TRI-reagent/Trizol (Sigma, St. Louis, MO, USA). Moloney-Murine Leukemia Virus reverse transcriptase (Invitrogen, Bleiswijk, The Netherlands) was used for cDNA synthesis. Real-time quantitative PCR was performed with the TaqMan™ master mix (Applied Biosystems, Vilnius, Lithuania) on a StepOnePlus™ Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). mRNA expression levels were calculated using relative standard curves and normalized to the expression of *Cyclophilin* as a housekeeping gene in both liver and ileum, and were further normalized to the mean of the respective control group. The sequences of Taqman primers and probes can be found in Supplemental Table S1.

2.6. Plasma parameters

Plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), and albumin levels were routinely determined using a Cobas 6000 analyzer with standard reagents (Roche Diagnostics, Rotkreuz, Switzerland).

Plasma lipoprotein profiles were determined after fractionation by fast protein liquid chromatography (FPLC) as described [14].

2.7. Statistics

Results in tables are presented as median and interquartile range, whereas figures are presented as Tukey box and whisker plots using GraphPad Prism8 (GraphPad Software, San Diego, CA). Multiple comparisons within genders were performed using the Kruskal-Wallis H test, followed by Conover *post-hoc* comparisons in Brightstat [19]. P values <0.05 were considered statistically significant.

3. Results

3.1. Morphometric parameters are not affected by OCA treatment in WT or *Cyp2c70*$^{-/-}$ mice

Our previous study [14] has shown that *Cyp2c70*$^{-/-}$ mice display ductular reactions and portal fibrosis by the age of 12 weeks, with more pronounced pathological features being observed in female than in male mice. To investigate the impact of OCA on existing cholangiopathy in *Cyp2c70*$^{-/-}$ mice, OCA was mixed into standard chow diet of male and female WT and *Cyp2c70*$^{-/-}$ mice (to receive ~10 mg/kg/day) between week 12 and 16 of age. The treatment and control groups were matched for baseline body weights within either gender and genotype (Table 1). OCA treatment did not impact body weight, food intake or feces production in WT and *Cyp2c70*$^{-/-}$ mice (Table 1). In line with our previous observations [14], female *Cyp2c70*$^{-/-}$ mice had larger livers than female WT mice. OCA treatment did, however, not impact liver weights in female *Cyp2c70*$^{-/-}$ mice. Liver weights of male mice were not impacted by *Cyp2c70*-deficiency or OCA treatment (Table 1).

3.2. OCA induces a more hydrophilic bile acid pool in WT mice but a more hydrophobic bile acid pool in *Cyp2c70*$^{-/-}$ mice

In line with the essential role of CYP2C70 in MCA production, these tri-hydroxylated BA species were absent in the plasma of *Cyp2c70*$^{-/-}$ mice, while being prominently present in WT mice (Fig. 1A, B). Total plasma BA concentrations were higher in female *Cyp2c70*$^{-/-}$ mice than in their WT littermates, but were not significantly impacted by OCA treatment in either genotype (Fig. 1C). No differences in plasma total BA concentrations were observed between the male groups (Fig. 1D). The concentrations of total OCA in plasma were significantly higher in both male and female *Cyp2c70*$^{-/-}$ mice compared to their WT controls (Fig. 1C, D). In plasma, OCA accounted for 17% (13%–20%) of the total plasma BAs in female WT mice receiving OCA, which was significantly higher than 8% (4%–13%) in female *Cyp2c70*$^{-/-}$ mice treated with OCA (Fig. 1A). OCA accounted for 19% (13%–27%) of total plasma BAs in both male groups receiving OCA (Fig. 1B). Female *Cyp2c70*$^{-/-}$ mice showed an elevated percentage of conjugated BAs in plasma compared to female WT controls, which was not impacted by OCA (Fig. 1C), while male *Cyp2c70*$^{-/-}$ mice showed an increase in conjugated BAs upon OCA treatment (Fig. 1D). Interestingly, OCA reduced the relative amounts of CA in plasma BAs in both WT and *Cyp2c70*$^{-/-}$ mice, while OCA increased MCAs and CDCA in WT and *Cyp2c70*$^{-/-}$ mice, respectively (Fig. 1A, B). Thus, OCA decreased the ratio of 12α- to non-12α-hydroxylated BAs in plasma in WT and *Cyp2c70*$^{-/-}$ mice of both genders (Fig. 1C, D).

Since bile ducts are exposed to biliary BAs, BA concentrations, composition and hydrophobicity were also analyzed in gallbladder bile. OCA treatment did not alter the biliary BA concentrations in mice

Table 1
Basal parameters in mice fed a chow diet with or without OCA treatment for 4 weeks.

	F-WT Control	F-WT OCA	F-KO Control	F-KO OCA	M-WT Control	M-WT OCA	M-KO Control	M-KO OCA
Baseline BW (g)	20.4 (18.7–20.8)	20.5 (20.1–20.9)	21.1 (17.9–21.6)	21.0 (19.8–21.3)	24.6 (24.6–25.0)	25.0 (23.6–25.1)	24.3 (23.6–24.8)	25.1 (24.0–25.3)
Final BW (g)	21.8 (20.5–22.1)	22.0 (21.2–22.2)	22.4 (20.6–23.5)	21.7 (21.2–23.0)	27.1 (25.4–27.1)	26.3 (24.7–27.3)	26.6 (25.6–27.0)	26.2 (25.0–27.6)
Food intake (g/day)	4.5 (4.3–4.6)	4.6 (4.2–4.9)	4.2 (4.2–4.3)	4.4 (4.4–4.5)	4.6 (4.4–4.6)	4.5 (4.1–4.6)	4.7 (4.3–4.9)	4.7 (4.7–4.9)
Liver weight/BW (%)	4.9 (4.8–5.0)	4.9 (4.7–5.0)	6.8### (6.3–8.3)	7.6### (6.3–8.5)	5.0 (4.7–5.3)	4.9 (4.8–5.3)	5.3 (5.1–5.5)	5.4 (5.2–5.5)
Feces output (g/day)	1.3 (1.2–1.4)	1.4 (1.3–1.4)	1.2 (1.2–1.5)	1.3 (1.2–1.5)	1.4 (1.4–1.4)	1.3 (1.2–1.5)	1.3 (1.3–1.4)	1.4 (1.3–1.5)

N = 7–11 mice/group. Values are presented as medians (the interquartile range). Comparisons were made within genders with Kruskal-Wallis H test, followed by Conover *post-hoc* comparisons. F, female; M, male; WT, *Cyp2c70*$^{+/+}$; KO, *Cyp2c70*$^{-/-}$; OCA, obeticholic acid; BW: body weight.
$P < 0.001$ in *Cyp2c70*$^{-/-}$ mice compared to WT receiving the same treatment.

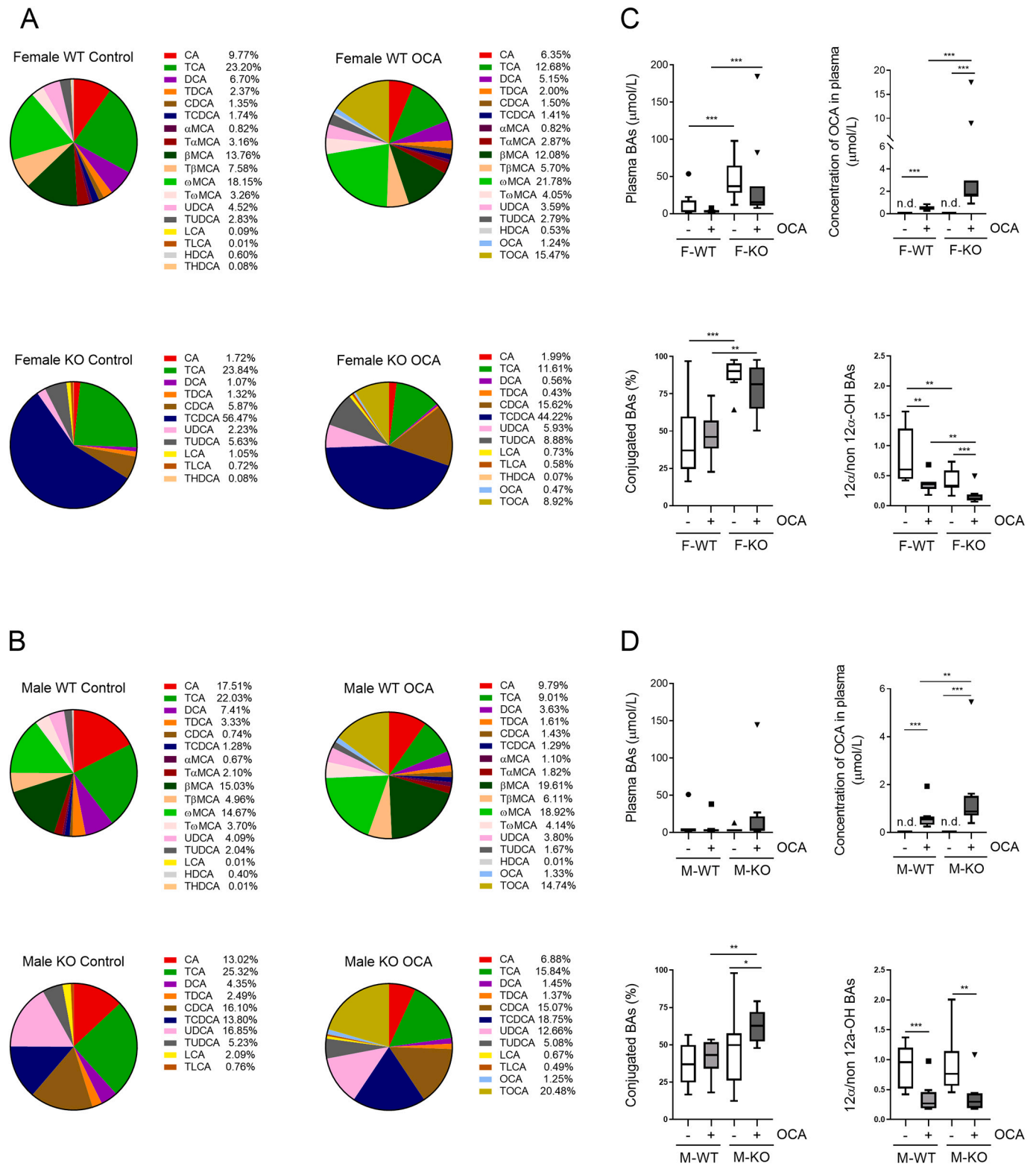

Fig. 1. Plasma OCA concentration is higher in *Cyp2c70*$^{-/-}$ mice compared to WT mice.
BA composition in female (A) and male (B) mice. BA and total OCA concentration, percentage of conjugated BAs in plasma and ratio of 12α- to non-12α-hydroxylated BAs of female (C) and male (D) mice. $N = 7$–11 mice/group. Data are presented as average percentage in pie charts and as Tukey box and whisker plots. *P* value represents * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ between groups as indicated. Kruskal-Wallis H test with Conover *post-hoc* comparisons was used to test significance of multiple comparisons. BA, bile acid; F, female; M, male; WT, *Cyp2c70*$^{+/+}$; KO, *Cyp2c70*$^{-/-}$; OCA, obeticholic acid.

(Supplemental Fig. S1). Similar changes as induced by OCA on BA composition in plasma, but of a different magnitude, were observed in bile of WT as well as of *Cyp2c70*$^{-/-}$ mice (Fig. 2A, B and Supplemental Tables S2 and S3). Specifically, bile of untreated *Cyp2c70*$^{-/-}$ mice showed a high abundance of CDCA compared to WT controls. The proportion of CA in biliary BAs was decreased in female *Cyp2c70*$^{-/-}$ mice compared to female WT, while it remained similar in male groups. Upon OCA treatment, the relative abundance of CA was significantly reduced in *Cyp2c70*$^{-/-}$ mice of both genders (Supplemental Tables S2 and S3). Importantly, OCA increased the percentages of MCAs in bile of WT mice, but caused a higher contribution of CDCA in bile of *Cyp2c70*$^{-/-}$ mice (Fig. 2A, B). Thus, the ratio of 12α- to non-12α-hydroxylated BAs in bile was decreased by OCA in WT and *Cyp2c70*$^{-/-}$ mice of both genders (Fig. 2A, B). OCA accounted for only 1–5% of the total biliary BAs in mice, with a larger enrichment in *Cyp2c70*$^{-/-}$ mice compared to WT groups. Based on the published values for HI of individual BAs [16], we calculated the HI for OCA and tauro-OCA (TOCA) and found that OCA was approximately as hydrophobic as lithocholic acid (LCA) (Fig. 2C). In terms of hydrophobicity, the increase of MCAs in bile of OCA-treated WT mice outweighed the impact of the hydrophobic OCA itself and, hence, made their BA pool even more hydrophilic, while the increase of CDCA and the presence of OCA in bile of OCA-treated *Cyp2c70*$^{-/-}$ mice led to a more hydrophobic BA composition in bile.

To understand the effects of OCA treatment on BA composition and transport pathways, we quantified expression of *Fxr/Nr1h4* and established FXR target genes in both liver and ileum. *Fxr/Nr1h4* and *Ostβ* expression showed a tendency to increase in the liver of OCA-treated female WT and *Cyp2c70*$^{-/-}$ mice compared to untreated female controls, while *Shp/Nr0b2* expression tended to increase in the liver of female *Cyp2c70*$^{-/-}$ mice upon OCA treatment (Fig. 3A). In the ileum, FXR downstream genes including *Shp*, *Ibabp/Fabp6* and *Fgf15* were significantly up-regulated by OCA in female *Cyp2c70*$^{-/-}$ mice but not in WT (Fig. 3B). Hepatic *Fxr* and *Shp* expression was not affected by OCA in male mice, but expression of *Ostβ* was significantly up-regulated by OCA in both male *Cyp2c70*$^{-/-}$ and WT mice (Supplemental Fig. S2A). Ileal expression of *Shp* was significantly increased in both male WT and *Cyp2c70*$^{-/-}$ mice as well (Supplemental Fig. S2B). Expression of *Ibabp/Fabp6* and *Fgf15* in the ileum were, however, not significantly changed upon OCA treatment in male mice of both genotypes. The gene encoding the rate-controlling enzyme in BA synthesis, i.e., *Cyp7a1* tended to be suppressed by OCA in female *Cyp2c70*$^{-/-}$ mice (Fig. 3A). *Cyp2c70*-deficiency considerably decreased *Cyp8b1* mRNA expression in livers of non-treated mice. However, despite the fact that the ratio of 12α- to non-12α-hydroxylated BAs in the circulating pool was significantly decreased upon OCA treatment in both female WT and *Cyp2c70*$^{-/-}$ mice (Figs. 1C, 2A), indicating reduced 12α-hydroxylation activity, significant down-regulation of hepatic *Cyp8b1* expression was only observed in female WT mice treated with OCA, while the reduction of *Cyp8b1* expression in OCA-treated compared to non-treated female *Cyp2c70*$^{-/-}$ mice did not reach statistical significance (Fig. 3A). In male mice, *Cyp8b1* expression was significantly down-regulated by OCA in both WT and *Cyp2c70*$^{-/-}$ mice (Supplemental Fig. S2A), in line with the marked reduction of the ratio of 12α- to non-12α-hydroxylated BAs in the circulating pool (Figs. 1D, 2B). The relatively small effects on hepatic and ileal expression of FXR target genes are in line with earlier mouse studies in which OCA was mixed in the diet (Supplemental Table S4), which might be related to dose and/or time of last food intake relative to that of organ collection. Importantly, however, daily fecal BA excretion, which reflects hepatic BA synthesis under steady conditions, was lower in female *Cyp2c70*$^{-/-}$ mice than that in female WT and was further decreased by OCA treatment in both genotypes (Fig. 3C), which confirms that FXR activation by OCA indeed reduced BA production. Inhibition of hepatic BA synthesis was also observed in male WT mice treated with OCA, while a similar trend was observed in male *Cyp2c70*$^{-/-}$ mice (Supplemental Fig. S2C). Decreased expression of *Ntcp/Slc10a1* (Fig. 3D), a transporter involved in BA uptake by hepatocytes from the blood compartment, might explain the higher plasma BA levels in female *Cyp2c70*$^{-/-}$ mice compared to female WT controls (Fig. 1C). Expression of *Ntcp* remained unchanged in female mice upon OCA treatment and was barely affected in OCA-treated male *Cyp2c70*$^{-/-}$ mice compared to untreated controls (Fig. 3D, Supplemental Fig. S2D). Expression of the canalicular BA transporter *Bsep/Abcb11* was also not altered upon OCA treatment in female and male mice of either genotype (Fig. 3D, Supplemental Fig. S2D). Collectively, OCA treatment resulted in uptake of the FXR agonist into the enterohepatic circulation of the mice, with a higher relative abundance in plasma than in bile, and resulted in variable induction of FXR target genes in ileum and livers but a clear suppression of daily BA synthesis demonstrating effectiveness of the treatment.

3.3. OCA does not alleviate cholangiopathy or hepatic fibrosis in *Cyp2c70*$^{-/-}$ mice

Next, we investigated whether OCA modulated liver pathology in *Cyp2c70*$^{-/-}$ mice. Because female but not male *Cyp2c70*$^{-/-}$ mice develop progressive cholangiopathy and liver fibrosis [14], the main focus in the next sections is on the female groups.

Histological examination of the livers confirmed that ductular reactions and collagen deposition were evident in livers of untreated female *Cyp2c70*$^{-/-}$ mice and to a lesser extent in male *Cyp2c70*$^{-/-}$ mice, as shown by H&E staining, CK19 immunohistochemistry (cholangiocytes) and Sirius Red staining (collagen deposition) of liver sections (Fig. 4A, B, C, E). OCA treatment did not improve liver histology in female nor in male *Cyp2c70*$^{-/-}$ mice (Fig. 4A, B, C, E). In line with the Sirius Red staining, hepatic hydroxyproline content was higher in untreated *Cyp2c70*$^{-/-}$ mice than in WT controls and confirmed the more advanced fibrosis in female compared to male *Cyp2c70*$^{-/-}$ mice. OCA treatment did, however, not impact the hepatic hydroxyproline content in *Cyp2c70*$^{-/-}$ mice of either sex (Fig. 4D).

Plasma AST and ALT levels were ~10-fold higher in untreated female *Cyp2c70*$^{-/-}$ mice compared to WT controls and were ~3-fold higher in male *Cyp2c70*$^{-/-}$ mice than in their WT littermates (Table 2). Plasma albumin was comparable among all four groups of both genders, indicating that *Cyp2c70*$^{-/-}$ mice maintained critical liver functions. OCA treatment did not impact on plasma transaminase levels in either gender of *Cyp2c70*$^{-/-}$ mice (Table 2).

3.4. Fibrogenesis, inflammation and cellular senescence in livers of female *Cyp2c70*$^{-/-}$ mice are not impacted by OCA treatment

In order to evaluate whether OCA has any dampening effects on fibrogenesis and inflammation that may be beneficial on the longer term, we quantified the hepatic expression of genes involved in matrix production and degradation, inflammation and cellular senescence, processes that have all been reported to play a role in cholangiopathies [20–22]. Compared to untreated female WT mice, pro-fibrotic markers *αSMA (Acta2)*, *Tgfβ1* and *Col1a1* were markedly up-regulated in livers of untreated female *Cyp2c70*$^{-/-}$ mice (Fig. 5A). Likewise, matrix metalloproteases (*Mmp12* and *Mmp13*) and tissue inhibitors of MMPs (including *Timp1* and *Pai1*) were increased in livers of untreated female *Cyp2c70*$^{-/-}$ mice (Fig. 5A), indicating augmented matrix remodeling in association with the hydrophobic BA pool in female *Cyp2c70*$^{-/-}$ mice. Expression of *Krt19*, a marker for cholangiocytes, was also significantly elevated in untreated female *Cyp2c70*$^{-/-}$ mice (Fig. 5B), in line with the CK19 staining in the liver (Fig. 4E). Expression of inflammatory markers (*Mcp1*, *Tnfα*) and senescent markers (*P16*^{INK4A}*/Cdkn2a*, *P21/Cdkn1a*) was also induced in livers of female *Cyp2c70*$^{-/-}$ mice compared to female WT controls (Fig. 5C, D), suggesting that inflammation and cellular senescence might play a role in the development of liver disease in these mice. Importantly, OCA treatment did not affect expression of any of these genes in livers of female *Cyp2c70*$^{-/-}$ mice (Fig. 5).

In males, *Col1a1* was moderately elevated in livers of *Cyp2c70*$^{-/-}$

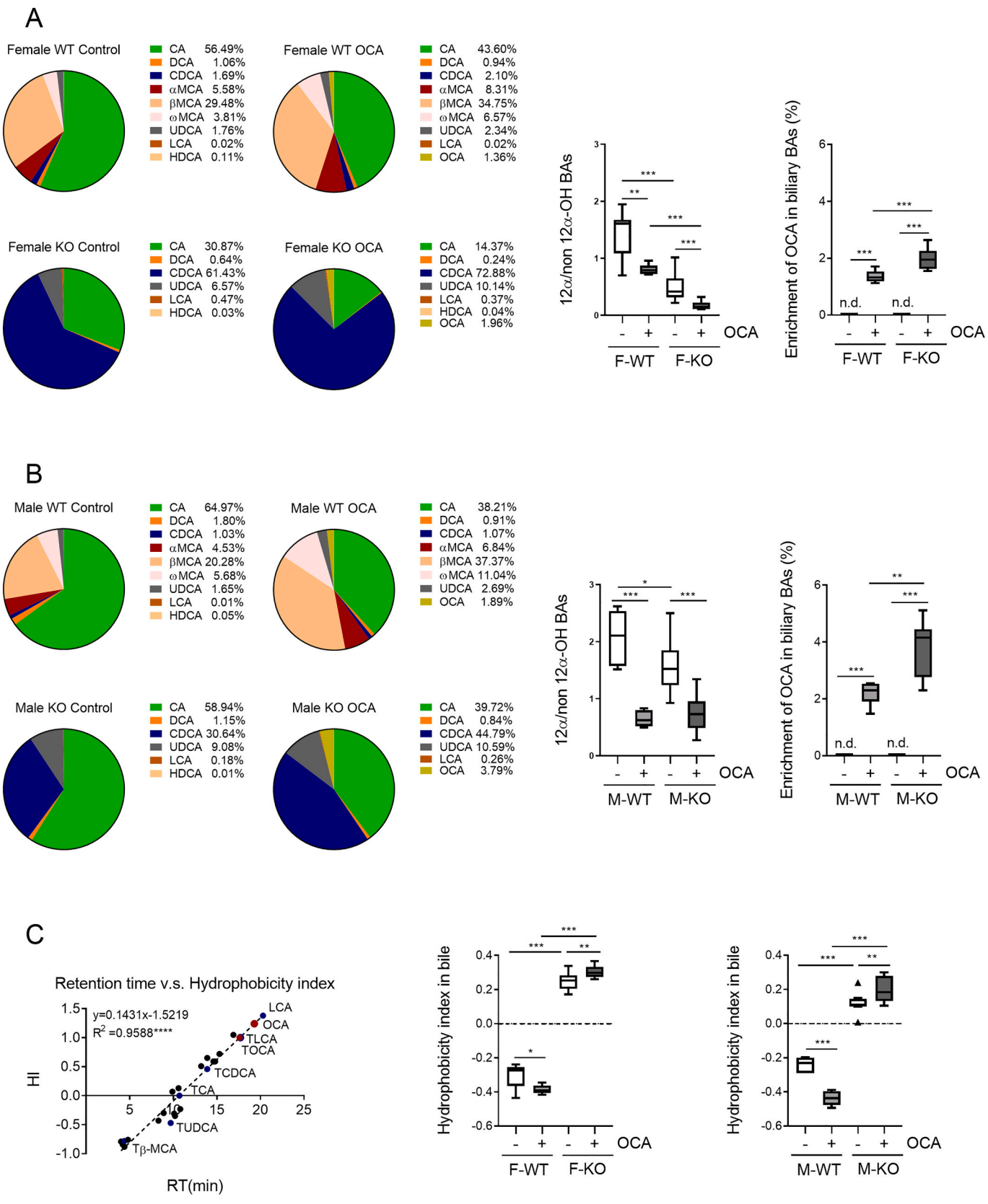

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Fig. 2. OCA induces a more hydrophilic BA composition in gallbladder bile of WT mice but a more hydrophobic composition in *Cyp2c70*$^{-/-}$ mice. (A) Total biliary BA composition and ratio of 12α- to non-12α-hydroxylated BAs in female mice. More than 99.5% of BAs were conjugated with taurine, while 100% of OCA in bile was taurine-conjugated. TOCA accounted for 1.1–2.6% of total biliary BAs in female mice receiving OCA treatment. (B) Total biliary BA composition and ratio of 12α- to non-12α-hydroxylated BAs in male mice. More than 99.5% of BAs were conjugated with taurine, while 100% of OCA in bile was taurine-conjugated. TOCA accounted for 1.5–5% of total biliary BAs in male mice receiving OCA treatment. (C) Retention time of BA species on UHPLC/MS plotted against the published value of their Heuman's index [16]. Calculated HIs for OCA and TOCA are 1.24 and 1.00, respectively, indicated by the red dots. HI of all biliary BAs combined was calculated based on [16]. $N = 7$–11 mice/group. Data are presented as average percentage in pie charts and as Tukey box and whisker plots. P values represent $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ between groups as indicated. Kruskal-Wallis H test with Conover *post-hoc* comparisons was used to test significance of multiple comparisons. BA, bile acid; F, female; M, male; WT, *Cyp2c70*$^{+/+}$; KO, *Cyp2c70*$^{-/-}$; HI, hydrophobicity index; OCA, obeticholic acid; TOCA, taurine- conjugated obeticholic acid; UHPLC/MS, ultra high-performance liquid chromatography tandem mass spectrometry. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

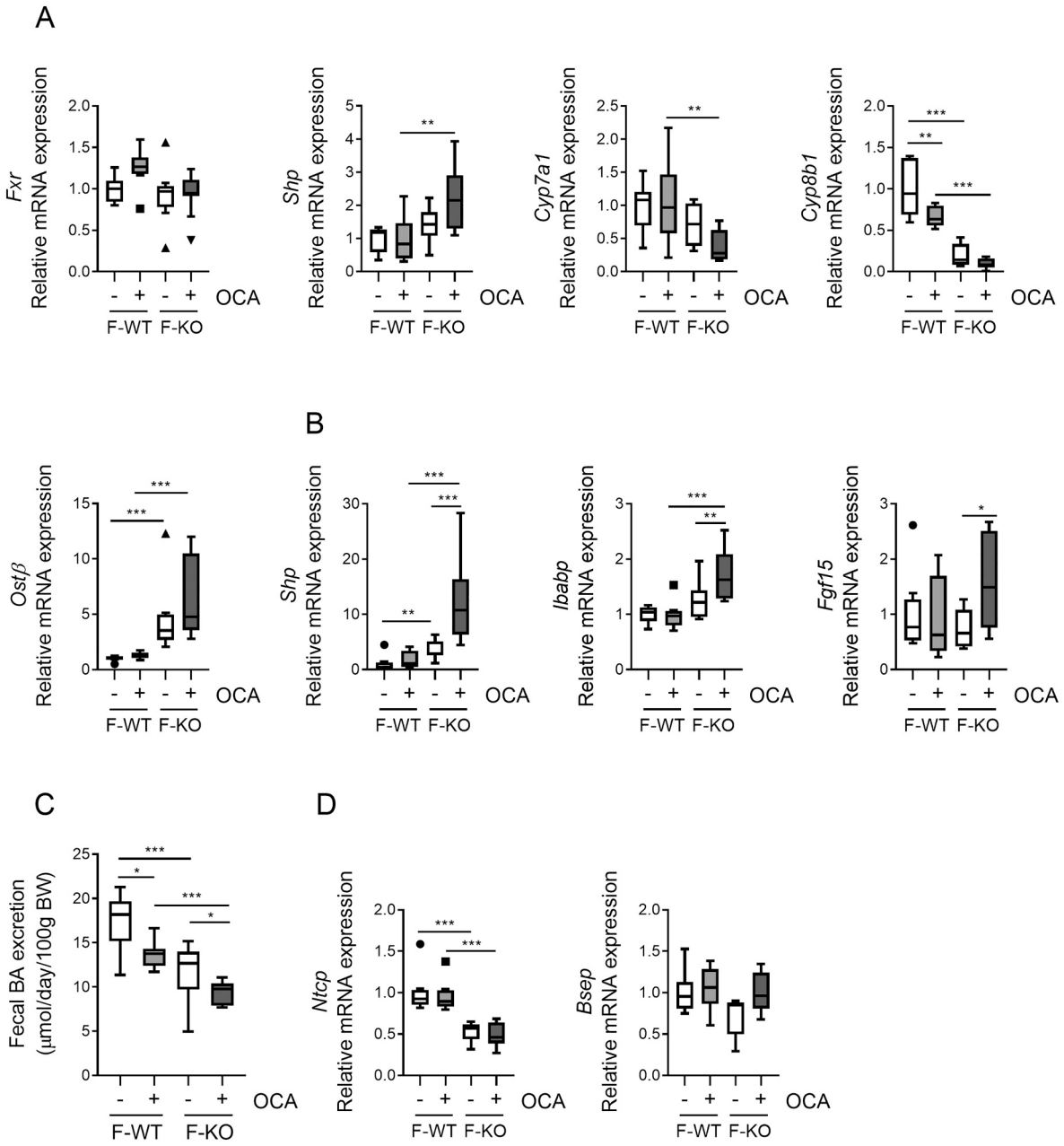
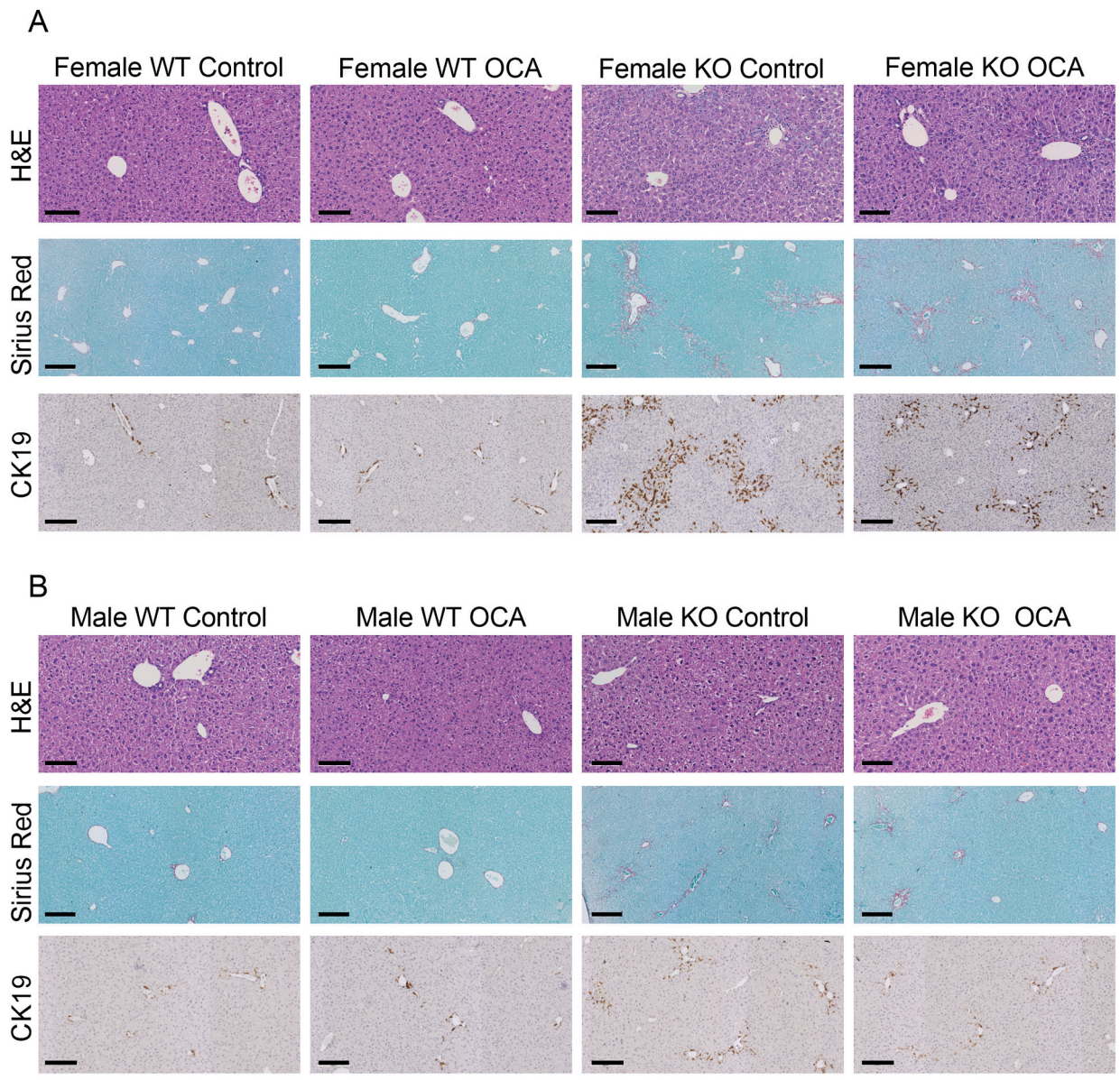
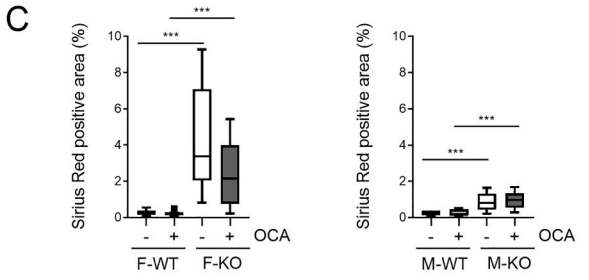
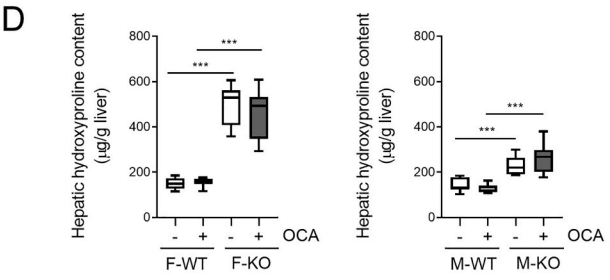


Fig. 3. OCA inhibits BA synthesis in female mice.
Female WT and Cyp2c70$^{-/-}$ mice were fed a chow diet with or without 10 mg/kg/day OCA for 4 weeks. Expression of *Fxr* and its target genes were quantified by RT-PCR in liver (A) and ileum (B). (C) Fecal BA excretion per 24 h. (D) Hepatic expression of BA transporters *Ntcp* and *Bsep*. $N = 8$–11 mice/group. Data are presented as Tukey box and whisker plots and P values represent $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ between groups as indicated. Kruskal-Wallis H test with Conover *post-hoc* comparisons was used for multiple comparisons. BA, bile acid; F, female; WT, *Cyp2c70*$^{+/+}$; KO, *Cyp2c70*$^{-/-}$; OCA, obeticholic acid; *Bsep*, bile salt export pump; *Cyp7a1*, cholesterol 7α-hydroxylase; *Cyp8b1*, sterol 12α-hydroxylase; *Fgf15*, fibroblast growth factor 15; *Fxr*, Farnesoid X receptor; *Ibabp*, ileal bile acid-binding protein; *Ntcp*, Na^{+}- taurocholate *co*-transporting polypeptide; *Ostβ*, Organic solute transporter beta; *Shp*, small heterodimeric partner.







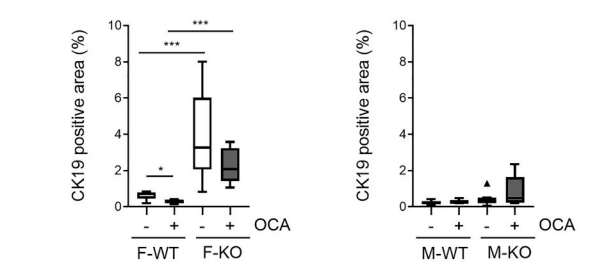


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Fig. 4. OCA does not impact biliary fibrosis in livers of *Cyp2c70*$^{-/-}$ mice.
Representative images of H&E staining (Scale bar: 100 μm), Fast Green/Sirius Red staining (Scale bar: 200 μm) and CK19 immunohistochemistry (Scale bar: 200 μm) in female (A) and male (B) mice. (C) Quantification of Sirius Red positive areas, (D) hepatic hydroxyproline contents determined by HILIC/MS analysis and (E) quantification of CK19 positive areas in female and male mice, respectively. $N = 7$–11 mice/group. Data are presented as Tukey box and whisker plots and P value represents * $P < 0.05$, *** $P < 0.001$ between groups as indicated. Kruskal-Wallis H test with Conover *post-hoc* comparisons was used for multiple comparisons. BA, bile acid; F, female; M, male; WT, *Cyp2c70*$^{+/+}$; KO, *Cyp2c70*$^{-/-}$; OCA, obeticholic acid; H&E, hematoxylin and eosin; CK19, cytokeratin 19; HILIC/MS, hydrophilic interaction liquid chromatography-mass spectrometry. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Plasma liver function markers in mice fed a chow diet with or without OCA treatment for 4 weeks.

	F-WT Control	F-WT OCA	F-KO Control	F-KO OCA	M-WT Control	M-WT OCA	M-KO Control	M-KO OCA
AST (U/L)	60 (56–63)	60 (49–75)	590 (488–1669) ###	750 (545–910) ###	65 (55–70)	55 (50–61)	163 (144–179) ##	148 (98–248) ###
ALT (U/L)	28 (24–31)	33 (24–35)	695 (593–1876)###	675 (600–960)###	40 (35–40)	35 (34–36)	133 (110–231)###	133 (91–243) ###
Albumin (g/L)	36 (34–37)	34 (34–35)	36 (33–39)	35 (33–38)	33 (32–35)	36 (33–38)	35 (34–37)	34 (33–35)

N = 7–11 mice/group. Values are presented as medians (the interquartile range). Comparisons were made within genders using the Kruskal-Wallis H test, followed by Conover *post-hoc* comparisons. ##. $P < 0.01$ and ###. $P < 0.001$ in *Cyp2c70*$^{-/-}$ mice compared to WT receiving the same treatment. F, female; M, male; WT, *Cyp2c70*$^{+/+}$; KO, *Cyp2c70*$^{-/-}$; OCA, obeticholic acid; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

mice (Supplemental Fig. S3A), in accordance with the lower grade of fibrosis in livers of male *Cyp2c70*$^{-/-}$ mice compared to females (Fig. 4C, D). Expression of *αSMA* and *Tgfβ1* was not affected by *Cyp2c70*-deficiency in male mice (Supplemental Fig. S3A). Yet, expression of *Mmp12*, *Mmp13* and *Timp1* was substantially up-regulated in male *Cyp2c70*$^{-/-}$ compared to WT mice. In addition, expression of *Krt19*, *Mcp1, Tnfα* and *P16*^{INK4A} tended to increase in male untreated *Cyp2c70*$^{-/-}$ mice compared to male untreated WT (Supplemental Fig. S3B–D). In keeping with the absence of effects of OCA in female mice, OCA treatment did also not alter expression of these genes in male *Cyp2c70*$^{-/-}$ mice (Supplemental Fig. S3). Therefore OCA does not appear to exert any effects on the increased fibrogenesis, inflammation or cellular senescence observed in the liver of *Cyp2c70*$^{-/-}$ mice.

3.5. OCA decreases plasma total cholesterol levels in female *Cyp2c70*$^{-/-}$ mice

Increased levels of plasma low-density lipoprotein cholesterol (LDL-C) and decreased plasma high-density lipoprotein cholesterol (HDL-C) were noticed as adverse effects of OCA in clinical trials [23,24], which raised the concern of the risk of atherosclerotic cardiovascular disease on the long term. Therefore, we also evaluated whether OCA impacted plasma cholesterol levels in this study. Total cholesterol levels in plasma were significantly higher in untreated female *Cyp2c70*$^{-/-}$ mice compared to their WT controls due to a ~3-fold increase of LDL-C levels without significant effects on HDL-C (Supplemental Fig. S4A). Hepatic expression of the sterol regulatory element-binding protein 2 (*Srebp2*), a sterol sensitive transcription factor, was not altered in female *Cyp2c70*$^{-/-}$ mice compared to WT nor was its target 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (*Hmgcr)*, encoding the rate-controlling enzyme in cholesterol synthesis (Supplemental Fig. S4B). However, expression of another *Srebp2* target gene, *Ldlr*, encoding the LDL receptor, was reduced in female *Cyp2c70*$^{-/-}$ mice compared to WT, providing a possible explanation to the increased LDL-C levels. Furthermore, hepatic expression of the oxysterol sensitive nuclear receptor, liver X receptor alpha (*Lxrα)* was slightly reduced as well in these mice (Supplemental Fig. S4B). OCA also reduced plasma total cholesterol levels in female *Cyp2c70*$^{-/-}$ mice, but significant effects on LDL-C and HDL-C or on expression of genes involved in cholesterol synthesis and transport were not observed (Supplemental Fig. S4). LDL-C levels were moderately increased in male *Cyp2c70*$^{-/-}$ mice (Supplemental Fig. S5A). However, OCA did not affect total cholesterol, LDL-C and HDL-C levels nor expression of genes involved in cholesterol synthesis and transport in male mice (Supplemental Fig. S5).

4. Discussion

In the present study, we demonstrate that activation of FXR by the steroidal agonist OCA does not translate into improvements of cholangiopathy and hepatic fibrosis in mice with a human-like BA composition, i.e., *Cyp2c70*$^{-/-}$ mice.

As previously described by us [14,25] and others [15,26], *Cyp2c70*$^{-/-}$ mice possess a human-like BA pool, lacking the hydrophilic, rodent-specific MCAs. Instead, these mice display an increased abundance of their precursor CDCA, which is a major constituent of the human BA pool, and its bacterial metabolite LCA. Young-adult *Cyp2c70*$^{-/-}$ mice show ductular reactions and biliary fibrosis, which is more pronounced in female than in male *Cyp2c70*$^{-/-}$ mice [14]. At more advanced ages, female *Cyp2c70*$^{-/-}$ mice develop progressive liver pathology with fibrosis, while liver pathology and fibrosis regress with age in male *Cyp2c70*$^{-/-}$ mice [14]. Although the origin of these gender differences remains to be fully established, it appears that the higher hydrophobicity of the female BA pool due to a strong suppression of *Cyp8b1* expression contributes to the more pronounced liver pathology in female *Cyp2c70*$^{-/-}$ mice. Accordingly, we found that treatment with UDCA, a hydrophilic BA, completely restored cholangiopathy, liver fibrosis and plasma transaminases levels in female *Cyp2c70*$^{-/-}$ mice [14]. Hence, female *Cyp2c70*$^{-/-}$ mice represent a new mouse model for cholangiopathies, that is responsive to the current first-line therapy for PBC. We tested whether the second-line therapy of PBC, OCA, also exerts beneficial effects in *Cyp2c70*$^{-/-}$ mice. Yet, although OCA treatment suppressed BA synthesis and increased BA hydrophobicity, it did not affect the cholangiopathy and hepatic fibrosis in Cyp2c70$^{-/-}$ mice. Interestingly, and in line with our observations in the current manuscript, absence of hepato-protective effects upon 4 and 6 weeks of OCA treatment has recently also been reported in the frequently used mouse model of PSC, i.e., *Mdr2*$^{-/-}$ (*Abcb4*$^{-/-}$) mice, in which hepatic fibrosis develops as a consequence of the absence of biliary phosphatidylcholine secretion [26,27].

In the current study, OCA had limited effect on hepatic FXR target genes in both *Cyp2c70*$^{-/-}$ and WT mice, with only an increased expression of *Ostβ* in male mice. OCA induced a small but significant elevation of intestinal FXR target gene expression in both male and female *Cyp2c70*$^{-/-}$ mice. OCA did induce intestinal expression of *Shp* in male WT mice but showed no effect on FXR target genes in female WT mice. Although this may appear surprising, it actually is not uncommon to see only a small or even no induction on hepatic FXR target genes when OCA is given by oral gavage or by mixing it into the diet of mice [28–30]. As summarized in Supplemental Table S4, the expression of FXR target genes in the liver might depend on the dose of OCA, way of administration and the timing of last does relative to harvest of organs

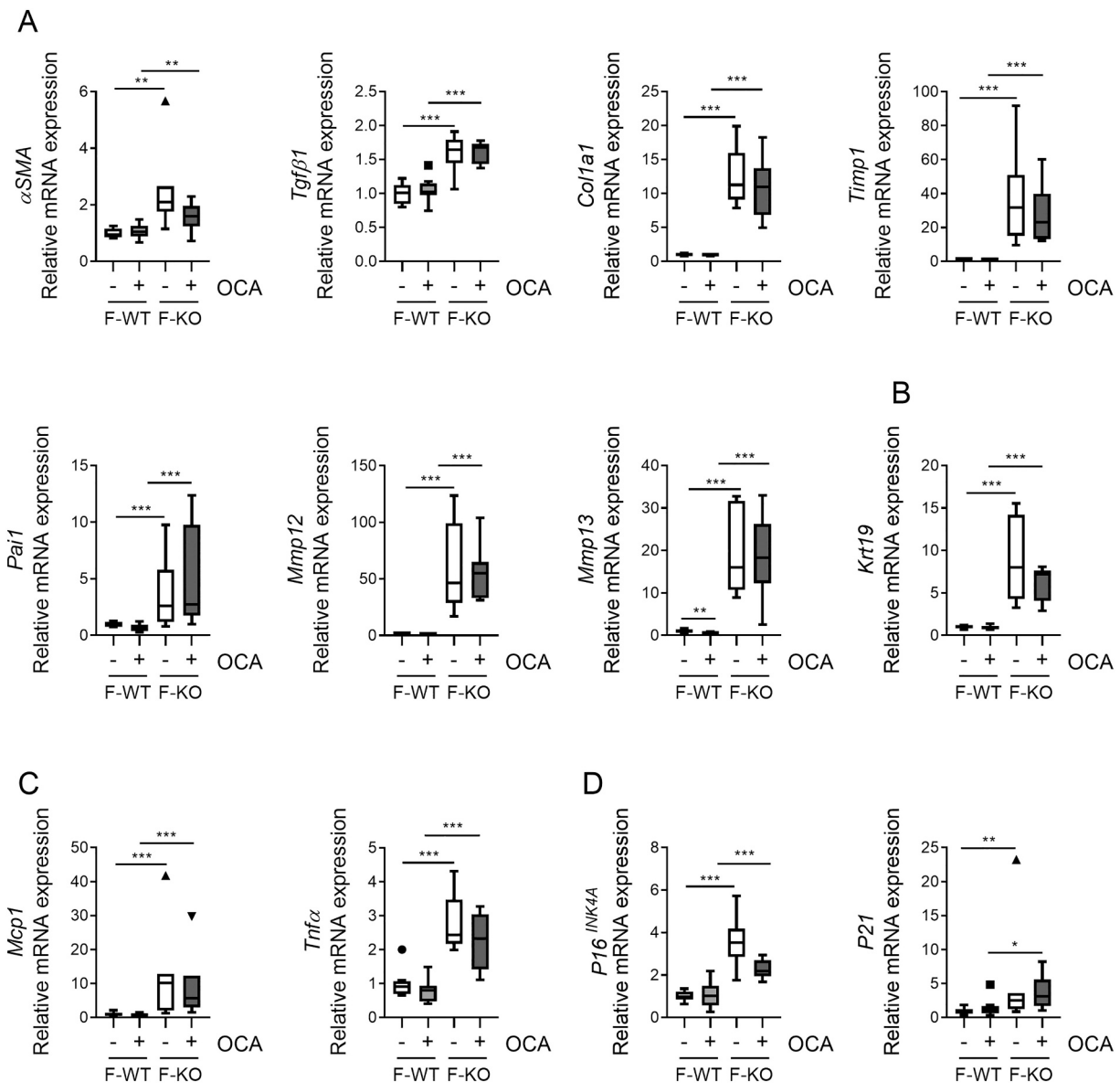

Fig. 5. OCA does not impact hepatic expression of genes involved in fibrosis, inflammation and cellular senescence in female *Cyp2c70*$^{-/-}$ mice.
Female WT and *Cyp2c70*$^{-/-}$ mice were fed a chow diet with or without 10 mg/kg/day OCA for 4 weeks. (A) Expression of genes involved in fibrosis; (B) Expression of *Krt19*; Expression of genes involved in inflammation (C) and cellular senescence (D). $N = 8$–11 mice/group. Data are presented as Tukey box and whisker plots and *P* values represent ** $P < 0.01$; *** $P < 0.001$ between groups as indicated. Kruskal-Wallis H test with Conover *post-hoc* comparisons was used for multiple comparisons. F, female; WT, *Cyp2c70*$^{+/+}$; KO, *Cyp2c70*$^{-/-}$; OCA, obeticholic acid; *αSMA*, alpha smooth muscle actin; *Col1a1*, collagen type I alpha 1 chain; *Krt19*, keratin 19; *Mcp1*, monocyte-chemoattractant protein 1; *Mmp12*, Matrix Metallopeptidase 12; *Mmp13*, Matrix Metallopeptidase 13; *P16*^{INK4A}, cyclin-dependent kinase inhibitor 2A (*Cdkn2a*); *P21*, cyclin-dependent kinase interacting protein 1 (*Cdkn1a*); *Pai1*, plasminogen-activator inhibitor 1; *Tgfβ1*, transforming growth factor beta 1; *Timp1*, metallopeptidase inhibitor 1; *Tnfα*, tumor necrosis factor α.

for analysis. In addition, expression of *Cyp8b1,* commonly modulated by FXR activation, was down-regulated by OCA in both male and female mice in our study. Accordingly, daily fecal BA excretion, reflecting daily BA synthesis, was significantly reduced upon OCA treatment in both *Cyp2c70*$^{-/-}$ and WT mice. Taken together, our data suggest that OCA treatment does activate FXR in our study.

We have previously shown that FXR activation by the pharmacological FXR agonist PX20606 leads to a marked increase in hydrophilicity of the BA pool in WT mice due to a strong suppression of *Cyp8b1* expression [18,31]. In mice lacking *Cyp2c70*, MCAs cannot be formed and FXR activation will lead to a higher proportion of hydrophobic CDCA and consequently to a more hydrophobic BA pool with a higher cytotoxic potential [18]. A similar increase in hydrophobicity of the human BA pool appears to occur upon FXR activation with OCA [32]. In the current study, OCA was also found to inhibit BA 12α-hydroxylation and, hence, to oppositely modulate BA pool hydrophobicity in *Cyp2c70*$^{-/-}$ and WT mice. Being a CDCA derivative with a hydrophobicity similar to that of LCA, the presence of OCA itself increased the hydrophobicity of the BA pool in *Cyp2c70*$^{-/-}$ mice and humans. Although this did not modulate indices of liver disease in our model, increased hydrophobicity of the circulating BA pool may be related to the toxicity and liver failure that have been observed upon excessive doses of OCA for prolonged periods of time, which raises particular concerns for cirrhotic patients with liver decompensation receiving OCA treatment [33]. It should be realized that, compared to OCA doses used in patients (10 mg/day), the 10 mg/kg/day OCA used in our mouse study was about 60-fold higher. However, after 3 weeks of treatment with 25 mg/day OCA in patients awaiting laparoscopic

cholecystectomy, OCA constituted 13.6% of the total biliary BAs [32], which is clearly higher than that in our mouse study (2.3% in bile on average). It should be realized that mice have a relatively larger BA pool size than humans (~800 μmoles/kg vs ~60 μmoles/kg, respectively) [14,34], which will affect enrichments of the respective BA pools with OCA. In plasma, concentration of OCA in our WT mice was quite comparable to that in patients [32], while both male and female *Cyp2c70*$^{-/-}$ mice had significantly elevated OCA levels compared to WT groups. This likely reflects less efficient hepatic uptake of OCA in *Cyp2c70*$^{-/-}$ mice due to low *Ntcp* expression.

As a second-line treatment for PBC patients, OCA has been reported to have anti-inflammatory and anti-fibrotic effects in in vitro and in vivo models [10,35,36,37]. Clinical trials have also demonstrated that OCA improved markers for liver injury and cholestasis in PBC and PSC patients [13,38]. One of the proposed protective roles of OCA in cholangiopathies is based on reduction of the toxic accumulation of BAs in the liver, which is supported by a study showing that OCA enhanced secretion of conjugated BAs from hepatocytes into bile canaliculi using positron emission tomography [8]. However, although multiple studies observed beneficial effects of OCA on fibrosis in NASH patients as evidenced by histological features in liver biopsies [23,39], clinical trials of OCA in cholangiopathies (including PBC and PSC) either had no fibrotic readouts [40] or reported no significant changes in fibrosis determined by enhanced liver fibrosis score (ELF) [12,41]. We investigated the impact of OCA on established, yet still progressing, cholangiopathy in *Cyp2c70*$^{-/-}$ mice of 12–16 weeks old [14]. In this treatment timeframe, OCA did not significantly impact plasma AST and ALT levels in *Cyp2c70*$^{-/-}$ mice nor modulate the mild fibrosis observed in male *Cyp2c70*$^{-/-}$ mice or the more advanced fibrosis present within the livers of female *Cyp2c70*$^{-/-}$ mice. This histological observation was confirmed by quantification of hepatic hydroxyproline contents. Moreover, hepatic expression of genes involved in fibrogenesis and matrix remodeling remained unaltered by OCA treatment. Expression of markers of cellular senescence, which has been reported to play important roles in cholangiopathies [20–22], also remained unchanged upon OCA treatment. Together, OCA treatment did not impact on any of the pathologic processes related to the cholangiopathy observed in *Cyp2c70*$^{-/-}$ mice.

Hydrophobic BAs have a greater cytotoxic potential and, hence, the hydrophobicity of biliary BAs is a critical contributor to the development of liver damage. OCA induced a more hydrophobic BA pool in *Cyp2c70*$^{-/-}$ mice of both genders, which did not translate into worsened liver phenotypes. The size of the BA pool may also impact liver physiology in *Cyp2c70*$^{-/-}$ mice. BA pool size was not quantified in the current study. However, we [14] have previously demonstrated that the BA pool size was significantly reduced in female *Cyp2c70*$^{-/-}$ mice compared to WT, while BA pool size remained unaltered in males upon *Cyp2c70*-ablation. Pharmacological FXR stimulation with OCA has been demonstrated to reduce BA pool size in WT mice [42]. It is therefore conceivable that OCA decreases BA pool size in our study as well. In addition, the concentrations to which BAs accumulate in intrahepatic bile ducts, which are likely relevant for the observed phenotype, are unknown and this is challenging to assess. In the current study, gallbladder BA concentrations (including OCA) were not reduced by OCA treatment, suggesting that biliary BA excretion was not largely suppressed by OCA. OCA also did not cause large effects on the expression of genes encoding BA transporters including *Ntcp* and *Bsep*. Potential long-term effects of OCA on liver damage and fibrosis in patients with PBC were assessed during the open-label extension of the POISE (the PBC OCA International Study of Efficacy) study. After 3-year of follow-up, OCA treatment was associated with improvement or stabilization of fibrosis compared to baseline [43]. Whether long-term OCA treatment of (female) *Cyp2c70*$^{-/-}$ mice will improve their liver phenotypes thus requires further investigation.

In summary, OCA treatment oppositely modulates BA pool composition in WT and *Cyp2c70*$^{-/-}$ mice due to preferential inhibition of the production of 12α-hydroxylated BAs, leading to increased relative amounts of hydrophilic MCAs in WT mice but more of the hydrophobic BA CDCA in *Cyp2c70*$^{-/-}$ mice. Despite a significant repression of BA synthesis, OCA did not improve cholangiopathy and biliary fibrosis in *Cyp2c70*$^{-/-}$ mice in this relatively short-term experimental set-up.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbalip.2022.159163.

CRediT authorship contribution statement

Rumei Li: Investigation, Formal analysis, Writing – original draft. **Milaine V. Hovingh:** Investigation. **Martijn Koehorst:** Investigation. **Pim de Blaauw:** Investigation. **Henkjan J. Verkade:** Writing – review & editing. **Jan Freark de Boer:** Supervision, Writing – review & editing, Funding acquisition. **Folkert Kuipers:** Supervision, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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