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FMO3 and its metabolite TMAO contribute to the formation of gallstones

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ABSTRACT

Trimethylamine-N-oxide (TMAO) is a metabolite derived from trimethylamine (TMA), which is first produced by gut microbiota and then oxidized by flavin-containing monooxygenase 3 (FMO3) in the liver. TMAO may contribute to the development of diseases such as atherosclerosis because of its role in regulating lipid metabolism. In this study, we found that high plasma TMAO levels were positively associated with the presence of gallstone disease in humans. We further found increased hepatic FMO3 expression and elevated plasma TMAO level in a gallstone-susceptible strain of mice C57BL/6J fed a lithogenic diet (LD), but not in a gallstone-resistant strain of mice AKR/J. Dietary supplementation of TMAO or its precursor choline increased hepatic FMO3 expression and plasma TMAO levels and induced hepatic canalicular cholesterol transporters ATP binding cassette (Abc) g5 and g8 expression in mice. Up-regulation of ABCG5 and ABCG8 expression was observed in hepatocytes incubated with TMAO in vitro. Additionally, in AKR/J mice fed a LD supplemented with 0.3% TMAO, the incidence of gallstones rose up to 70% compared with 0% in AKR/J mice fed only a LD. This was associated with increased hepatic Abcg5 and g8 expression induced by TMAO. Our study demonstrated TMAO could be associated with increased hepatic *Abcg5/g8* expression, biliary cholesterol hypersecretion and gallstone formation.

1. Introduction

Trimethylamine-N-oxide (TMAO) is derived from dietary choline, L-carnitine, and phosphatidylcholine [1]. These trimethylamine-containing compounds are metabolized by gut microbiota to produce an intermediate compound known as trimethylamine (TMA). After absorption, TMA is further metabolized to TMAO by flavin-containing monooxygenases (FMO) in the liver [2]. FMOs contain five functional FMO genes and they can catalyze a wide array of chemicals, including drugs, environmental pollutants, and dietary-derived compounds [3,4]. FMO3 is the key enzyme in the conversion of TMA to TMAO in the liver [5–7]. FMO3 mutation or deficiency can cause the inherited fish-odor syndrome, which is characterized by a peculiar body odor resulting from the failure to N-oxidize TMA [8].

An increasing amount of epidemiological evidences has identified the TMA–FMO3–TMAO pathway that regulates lipid metabolism in the body [9,10]. Plasma TMAO levels were shown to be positively associated with an increased prevalence of cardiovascular disease, atherosclerosis, and metabolic syndrome through its effects that cause lipid metabolism disorders [10–12].

Gallstone disease (GS) is a common disease throughout the world. It is highly prevalent in Western countries, where it affects over 10% of the population [13], and in China in the last decades [14] with the rapid development of the economy and westernization of life styles and dietary habits. Most of the gallstones in the gallbladder are the cholesterol type [15]. The pathogenic mechanism of gallstone formation is multifactorial, involving supersaturation of biliary cholesterol, impairment of gallbladder motility, hypersecretion of mucins, and gallbladder inflammation [16–20]. Among these, cholesterol supersaturation in bile plays a crucial role in promoting cholesterol gallstone formation [20] such as via enhanced intestinal cholesterol absorption [20], increased hepatic cholesterol uptake via scavenger receptor B type I (SRB1) [21], and canalicular secretion via ATP binding cassettes (ABC) G5 and G8 [22].

Whether TMAO leads to metabolic disorders that are associated with gallstone formation is unknown. In this study, for the first time, we

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found a strong positive relationship between the plasma TMAO level and gallstone disease in the population. To uncover the underlying mechanisms for how TMAO contributes to the development of gallstones, we showed that plasma TMAO and hepatic FMO3 expression were higher in a gallstone-susceptible strain of C57BL/6J mice compared with a gallstone-resistant strain of AKR/J mice. Lithogenic diet (LD) supplementation with TMAO increased incidence of gallstone in AKR/J mice which was associated with an up-regulation of hepatic ABCG5/G8 expression and cholesterol secretion into bile.

2. Materials and methods

2.1. Human study

The first cohort included 573 subjects who were confirmed to have gallstones by B-type ultrasonography (male/female: 216/357, GS group) during health examination and 565 subjects who were free of gallstone as controls (male/female: 215/350, GSF group). In the second cohort, 139 patients with cholesterol gallstone and 38 patients with pigment stone who underwent elective laparoscopic cholecystectomy were included. The gallstones were classified as typical cholesterol or pigment stones by visual inspection and when necessary, by analysis in the laboratory [23,24]. Ninety-nine patients who underwent elective operation because of diseases such as hernia, varicosis of great saphenous vein, lipoma, etc., were included as controls. All these controls were proved to be gallstone-free by B-type ultrasonography. None of the subjects included in this study was diagnosed as diabetes mellitus, disorders of thyroids or other disorders of endocrine system, morbid obesity or on anti-dyslipidemia treatment. Subjects who were on antibiotics treatment in the past 3 months were not included in the study. Fasting blood was obtained from each subject between 8: 00–9:00 am. The study protocol was approved by the Ethics Committee at Nanjing Medical University and at Shanghai East Hospital.

2.2. Animals study

Adult male C57BL/6J mice (8-week old, Shanghai SLAC Laboratory Animal Co., Ltd. Shanghai, China) and AKR/J mice (Jackson Laboratory, USA) were fed with a standard chow diet or a lithogenic diet (LD, containing 1.25% cholesterol and 0.5% cholic acid) for 8 weeks (n = 10 mice/group). In the TMAO supplementary feeding study, adult male AKR/J mice were fed with LD or LD + TMAO (0.3% in drinking water) for 8 weeks (n = 10 mice/group). In the TMAO dose-dependent feeding study, adult male C57BL/6J mice were fed with a diet containing 1% cholesterol supplemented with 0%, 0.12% or 0.3% TMAO in the drinking water until 8 weeks (n = 10 mice/group). After 4-week feeding, bile duct cannulation was performed in a set of mice according to the procedure as described [25]. In brief, common bile duct of mice fasted overnight was ligated and the common bile duct was cannulated with a PE-10 polyethylene catheter below the entrance of the cystic duct. The cystic duct was doubly ligated and a cholecystectomy was performed and the first hour hepatic bile were collected. In the choline fed study, adult male C57BL/6J mice were fed with diet containing 1% cholesterol with or without choline in diet (1%, w/w) for 8 weeks (n = 10 mice/group). Before sacrifice, mice were fasted overnight and on the day of sacrifice, blood samples were collected, liver and gallbladder were harvested. The study protocol was approved by the Ethics Committee at Nanjing Medical University and at Shanghai East Hospital.

2.3. Cell culture

Primary hepatocytes from C57BL/6J mice were isolated and culture as previously described [26]. The human hepatoma cell line, HepG2 was purchased from ATCC (HB-8065, Manassas, VA, USA) and human liver cell line L02 was obtained from Shanghai Institute of Biochemistry and Cell biology. Cells were cultured in DMEM with 10% FBS and 1% of antibiotic-antimycotic medium (Sigma-Aldrich, St. Louis, MO, USA) at 37 °C and 5% CO2. Cells were plated in 6-well plates. When 70–80% confluence was reached, TMAO was added at concentrations of 0, 50 and 250 μM, respectively. After a 24-hour culture, cells were collected. The experiments were repeated for three to four times.

2.4. Determination of TMAO concentrations

2.4.1. Pre-processing of plasma samples

Plasma or liver homogenates (20 μL) was mixed with 80 μL of 10 μM internal standard comprising d9-TMAO in methanol. Protein in the samples was precipitated by vortexing for 1 min and then the supernatant was recovered following centrifugation at 20,000*g* at 4 °C for 10 min.

2.4.2. Liquid chromatography-mass spectrometry/mass spectrometry measurement

The analysis was performed using a Waters ACQUITY™ Ultra Performance Liquid Chromatography system coupled to a Waters Quattro Premier mass spectrometer. Supernatant (10 μL) was separated by a HILIC Column (2.1 × 100 mm, 1.7 μm, Waters, USA) using a gradient elution with 10 mM ammonium acetate in water (A) and acetonitrile at a flow rate of 0.4 mL/min. The final LC gradient was as follows: 0–1.0 min 5% A, 1.0–1.5 min to 30% A, a 1.5–5 min hold at 30% A, 5–6 min 5% A, and then a 6–10 min hold at 5% A. TMAO and d9-TMAO were monitored using electrospray ionization in the positive-ion mode with multiple reaction monitoring (MRM) of parent and daughter-ion transitions of m/z 76 → 58 and 85 → 66, respectively. The parameters for the ion monitoring were as follows: cone, 30 V and collision, 25 V.

2.4.3. Analysis of biliary lipids

Total bile acids, phospholipids and cholesterol concentration in gallbladder or hepatic bile were measured by an enzymatic methods as previously described [27] using commercial purchased kits (cholesterol: Cat No.11491458216, from Roche Diagnostics GmbH, Germany; total bile acids: Cat No.BI2672 from RANDOX, UK; and phospholipids: phospholipid LabAssay kit, Cat No. WAKO 296-63801, from Fuji Film WAKO Pure Chemical Co, Japan). The relative concentrations of biliary lipids were expressed as molar percentages of the total biliary lipids. The cholesterol saturation was calculated according to Carey's critical table [28]. Bile acids profiles in gallbladder bile were analyzed on an Acquity UPLC system coupled to a Waters Xevo TQ-S MS (Waters, Manchester, UK) using the procedure as previously described [29]. Hydrophobicity index of bile acids was calculated according to the Heuman's method [30].

2.4.4. Determination of mRNA expression of genes involved in cholesterol metabolism using quantitative real-time PCR

Total RNA from liver tissue or hepatocytes was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). cDNA was synthesized with PrimeScript™ RT Master Mix (Takara, Dalian, China). Quantitative real-time PCR with SYBR Green was performed with RT-PCR LC480 II (Roche Diagnostics Ltd., Forrentrasse CH-6343 Rotkreuz, Switzerland) at final reaction volumes of 10 μL. The relative mRNA expression level was calculated by the $2^{-\Delta\Delta Ct}$ method using GAPDH as the internal control. The primer information for the genes is listed in Supplementary Table 1.

2.4.5. Detection of proteins involved in cholesterol metabolism by Western blot

Total proteins of homogenates of liver tissue or hepatocytes were separated on 10% SDS-PAGE gel, and then transferred onto polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA). After blocking with 5% non-fat dry milk in TBST, the membranes were

incubated with primary antibodies for FMO3 (Abcam Co., Cambridge, UK), SRB1 (Abcam), LDLr, ABCG5, and ABCG8 (Santa-Cruz Inc., CA, USA) overnight at 4 °C. The immune complexes were visualized with enhanced chemiluminescence (Millipore, Billerica, MA, USA). Anti-GAPDH (Beyotime, 1:1000) was performed as an internal control. Image Lab software (Bio-Rad Laboratories, Hercules, CA, USA) was used to quantify the band. Each experiment was performed at least twice.

2.5. Statistical analysis

All data were presented as the mean ± standard error of the mean (SEM). The variables were analyzed using an unpaired Student's *t*-test between two groups and an analysis of variance (ANOVA) for comparisons among three groups. Logistic regression analysis was used to explore the association between TMAO concentrations and gallstone disease. Covariates considered for adjustment in the logistic regression analysis included age, sex, and BMI. $P < 0.05$ (at two sides) was designated as statistically significant. All the statistics were performed using SPSS 20.0.

3. Results

3.1. Plasma TMAO is strongly associated with the incidence of gallstone disease in humans

Demographic characters of the human subjects were listed in Supplementary Table 2 and 3. In the total subjects, plasma TMAO was 398 ± 20 ng/mL in GS group and 260 ± 11 ng/mL in GSF group ($P < 0.01$, Fig. 1A). When the subjects were classified into normal weight or overweight, the difference between subjects with gallstone and without gallstone remained (Fig. S1A and B). The difference was also present independent of gender (Fig. S1C and D). In the second cohort, plasma TMAO level was further shown to be significantly higher in patients with cholesterol gallstone (390 ± 33 ng/ml) than in patients with pigment stone (221 ± 22 ng/ml) or in patients without gallstone (244 ± 18 ng/ml, Fig. 1B). No difference existed between patients with single stone and multiple stones (Fig. S1E).

Logistic regression analyses indicated a strong positive correlation between plasma TMAO and the presence of gallstone disease (Fig. 1C). After quadratic stratification of the plasma TMAO levels, the calculated odd ratio(OR) between TMAO and gallstone disease were in-gradient increased for the 2nd quartile (OR = 1.712, 95%CI: 1.167, 2.511), 3rd quartile (OR = 2.329, 95%CI: 1.6, 3.39) and 4th quartile (OR = 3.546, 95%CI: 2.485, 5.061). After adjustment for potential confounders, such as age, sex and BMI, the association was still present with OR = 1.723 (95%CI: 1.008, 2.945), 1.806(95%CI: 1.063, 3.068) and 2.607 (95%CI:

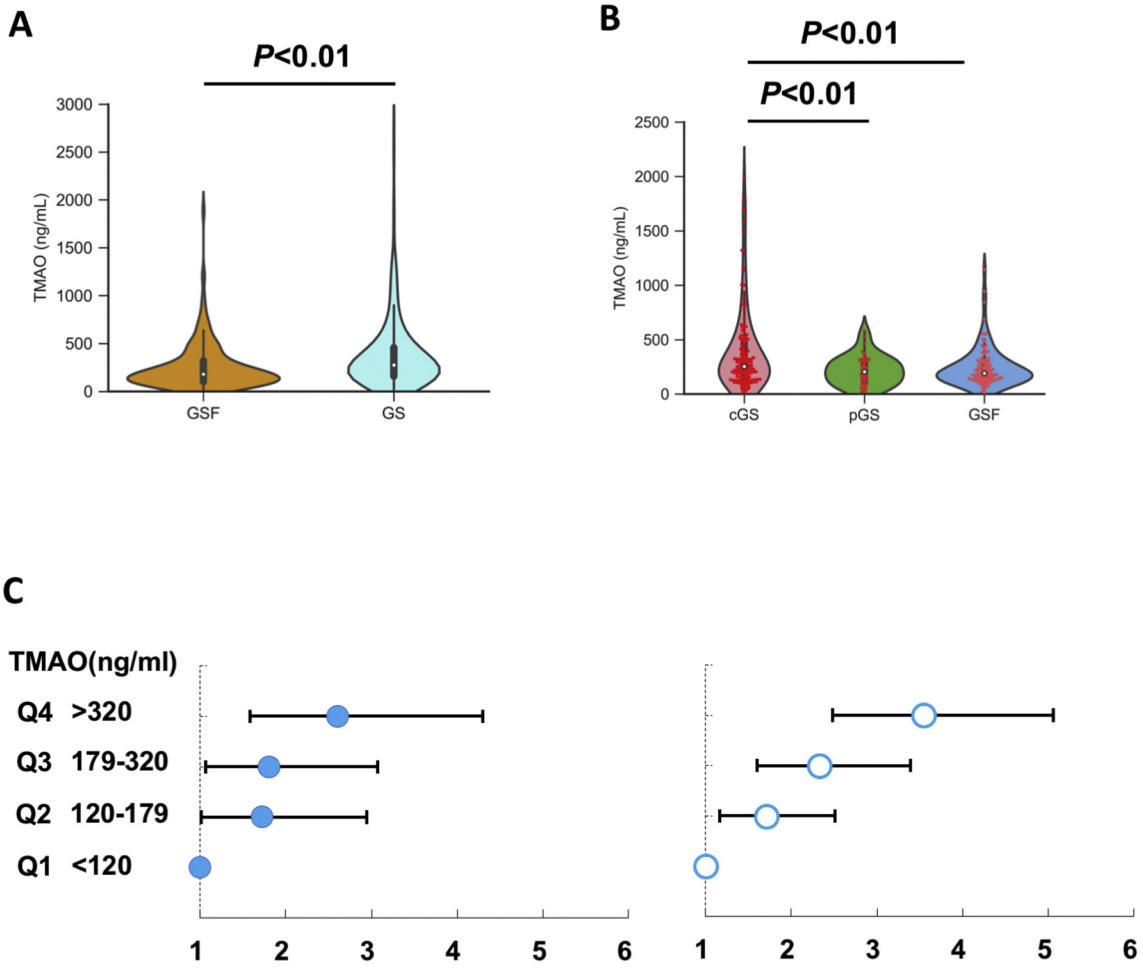

Fig. 1. Plasma TMAO was associated with gallstone disease in humans. (A). The plasma TMAO level between gallstone patients (GS) and gallstone-free controls (GSF) in all subjects. (B). The plasma TMAO level among patients with cholesterol gallstone (cGS), pigment stone (pGS) and gallstone-free control patients (GSF). (C). Odds ratios and 95%CI values for each quartile of plasma TMAO in association with the risk of gallstone disease using Logistic regression analysis (right panel) and after adjustment (left panel) for sex, age, and BMI.

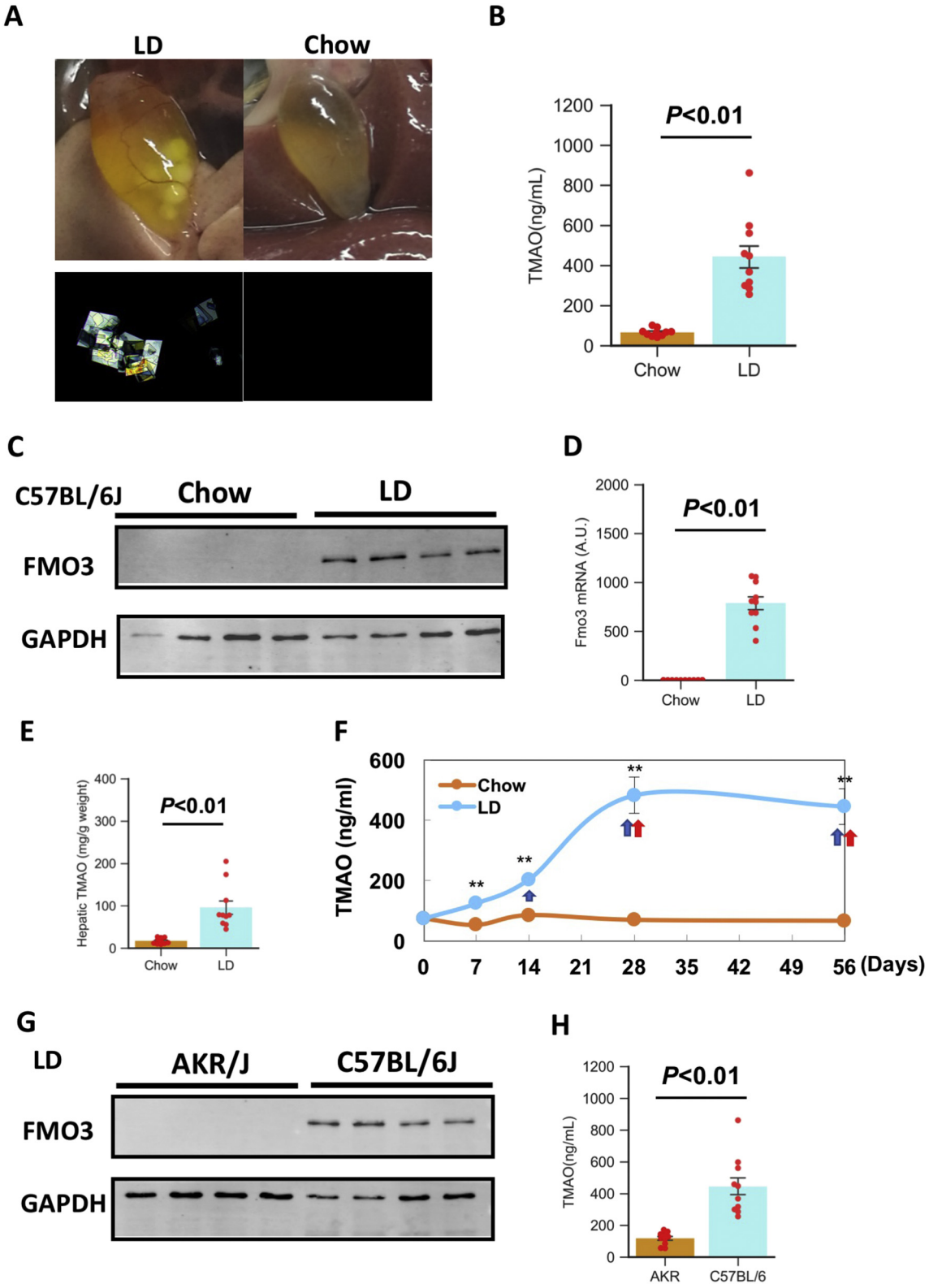

Fig. 2. Changes of hepatic FMO3 expression and plasma TMAO in mice fed with lithogenic diet. (A) Occurrence of gallstones in the gallbladder (upper) and cholesterol crystals in gallbladder bile under polarized microscopy (lower) in C57BL/6J mice fed a chow diet or a lithogenic diet (LD). (B) Plasma TMAO levels in C57BL/6J mice fed a chow diet or LD. (C) FMO3 protein and (D) Fmo3 mRNA expression in the livers of C57BL/6J mice fed a chow diet or LD. (E) Hepatic TMAO level in C57BL/6J mice fed a chow diet or LD. (F) Plasma TMAO level in C57BL/6J mice fed a chow diet or LD at Day 0, 7, 14, 28 and 56. ' ** ' represents $P < 0.01$ when LD compared with chow diet. Blue arrow indicated the occurrence of cholesterol crystals in gallbladder bile and red arrow indicated occurrence of gallstones in gallbladder. (G) Hepatic FMO3 protein expression and (H) plasma TMAO levels in C57BL/6J and AKR mice fed a LD. Data are expressed as the mean ± SEM.

1.581, 4.298) for 2nd, 3rd, and 4th quartile, respectively.

3.2. Induction of FMO3 expression and its metabolite, TMAO, in association with gallstone formation in C57BL/6J mice, but not in AKR/J mice

C57BL/6J is a gallstone-susceptible strain of mice with a gallstone incidence > 75% when fed a LD. However, AKR/J mice are a gallstone-resistant strain, and the incidence of gallstones is < 15% [31]. An 8-week LD resulted in gallstone formation in all C57BL/6J mice (100%, Fig. 2A). Very interestingly, plasma TMAO increased about 8-fold in C57BL/6J mice fed with LD compared with chow diet (Fig. 2B). This was due to dramatic induction of hepatic FMO3 expression (protein: Fig. 2C and mRNA: Fig. 2D) which catalyzing gut derived TMA to circulating TMAO. Hepatic TMAO level also increased significantly in C57BL/6J mice fed with LD (Fig. 2E). Plasma TMAO induced 230% as early as 7 days after LD when no cholesterol crystal was observed in gallbladder bile and continued to climb up to 6.9-fold until 28-day after diet and thereafter (Fig. 2F). In contrast, no gallstone (0%) or cholesterol crystal was found in AKR/J mice even at 56 days after LD. Hepatic FMO3 was resistant to be upregulated in AKR/J mice under LD (Fig. 2G), and plasma TMAO slight increased, which was 76% higher than that in chow-fed C57BL/6J mice (Fig. 2H).

3.3. TMAO and its precursor choline induced canalicular cholesterol transporters in the mouse liver

We fed C57BL/6J mice with 1% cholesterol diet supplementary with or without choline, a precursor for TMAO production. Dietary supplement with choline increased hepatic FMO3 protein levels (Fig. 3A), and accordingly, plasma TMAO level increased about 4-fold (Fig. 3B). Very interestingly, when measuring the mRNA expression of key genes for cholesterol uptake and secretion, we found increased expression of *Abcg5*, *g8* and *Ldlr* in liver by choline diet (Fig. 3C). There was no difference in bile acid composition of gallbladder bile between groups (Fig. S2A), nor in hydrophobicity index of bile acids (Fig. S2B).

Next, we fed C57BL/6J mice with 1% cholesterol diet and supplemented with low dose (0.12%) or high dose (0.3%) TMAO. Both the mRNA expression (Fig. 4A) and protein levels (Fig.4B and C) of canalicular cholesterol transporters Abcg5 and Abcg8, Ldlr and Srb1 significantly elevated by TMAO, especially at the high dose. Hepatic secretion of cholesterol was induced especially by 0.3% TMAO (Fig.4D). However, bile salts and phospholipids secretion were not affected by TMAO supplementation. No pathological difference in liver tissue was observed among groups (Fig. S3). In gallbladder bile, both 0.12% and 0.3% TMAO increased cholesterol molar% and CSI in gallbladder bile and the effect was more prominent at high dose (Fig.4E). (See Fig. 4.)

3.4. TMAO influenced expression of genes involved in cholesterol metabolism in hepatocytes

To explore the direct effect on cholesterol metabolism by TMAO, mouse primary hepatocytes cells were prepared and incubated with increase amount of TMAO at 0, 50 and 250 μM. As expected, TMAO dose-dependently significantly induced the mRNA expression of *Abcg5, Abcg8*, *Srb1* and *Ldlr* (Fig. 5A). The differences were further confirmed at the protein level (Fig. 5B). Similar results were obtained in human liver cells, L02 cells (Fig. S4), as well as in human hepatoma cells, HepG2 (Fig. S5). The effect of TMAO on hepatocytes was more prominent at a concentration of 250 μM.

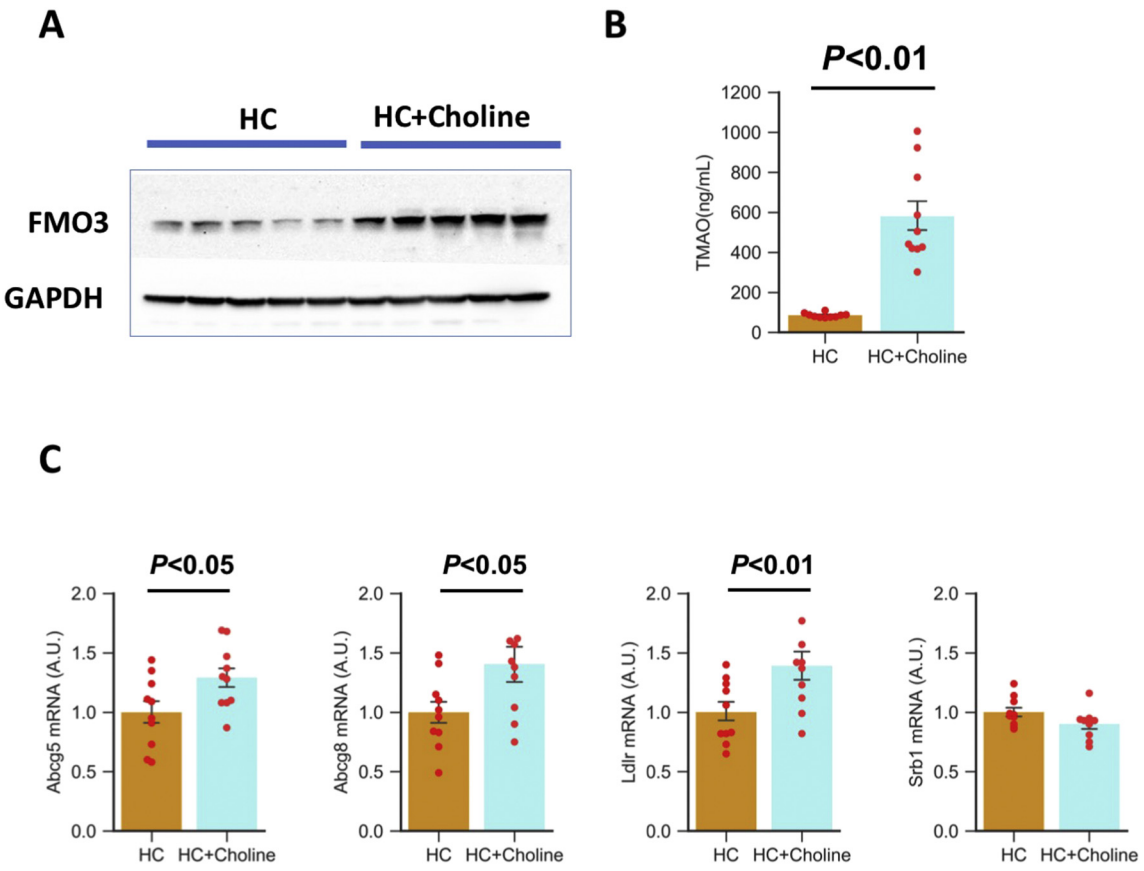

Fig. 3. Hepatic gene expression and plasma TMAO in mice fed a 1% cholesterol diet with/without 1% choline. (A) Hepatic FMO3 protein expression between groups. (B) Plasma TMAO levels between groups. (C) Hepatic mRNA expression of genes involved in cholesterol metabolism between groups. Data are expressed as the mean ± SEM. HC: high cholesterol diet

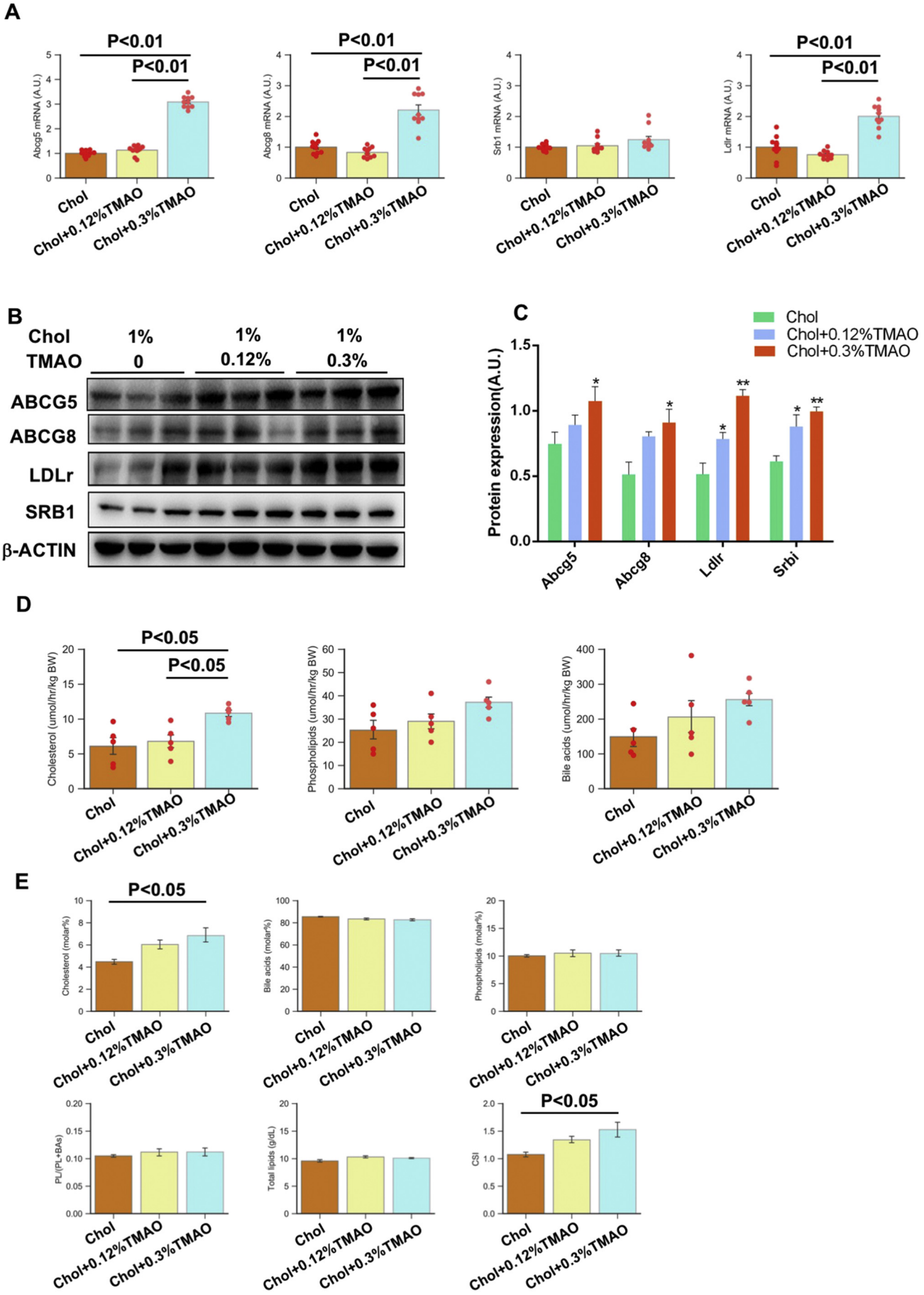

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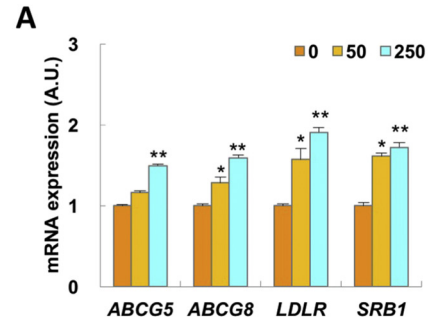
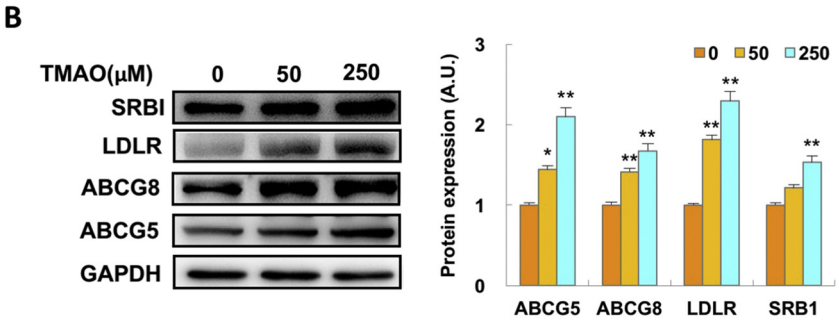
Fig. 4. Dose-dependent effect on hepatic cholesterol metabolism in mice fed a 1% cholesterol diet supplemented with different doses of TMAO. Changes in mRNA (A) and protein (B, C) expression of genes involved in hepatic cholesterol metabolism in mice that received different amount of TMAO in diet. '*' represents $P < 0.05$; and '**' represents $P < 0.01$. (D) Hepatic secretion of cholesterol, bile acids, and phospholipids in mice fed with different diets. (E) Biliary lipid composition of gallbladder bile in each group of mice. Data are expressed as the mean ± SEM. Chol: cholesterol diet





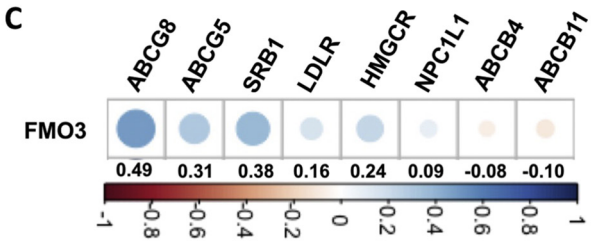


Fig. 5. Changes in gene expression in mouse primary hepatocytes treated with TMAO. (A) mRNA expression of genes involved in cholesterol metabolism in mouse primary hepatocytes treated with different concentrations of TMAO (0, 50, 250 μM). (B) ABCG5, G8, SRBI, and LDLR protein expression in mouse primary hepatocytes treated with TMAO. Data are expressed as the mean ± SEM. '*' represents $P < 0.05$; and '**' represents $P < 0.01$. (C) Associations between FMO3 mRNA expression and genes involved in hepatic cholesterol metabolism in patients with cholesterol gallstone. mRNA expression of genes were determined using real-time quantitative PCR in liver biopsies from patients with cholesterol gallstone disease that were collected during laparoscopic cholecystectomy (n = 37).

Furthermore, we measured the expression levels of FMO3 and key enzymes in cholesterol metabolism in liver biopsy samples from patients with cholesterol gallstone disease. As expected, positive correlations between *FMO3* and *ABCG5/G8* and *SRB1* expressions were present in human liver in vivo (Fig. 5C).

3.5. Dietary supplement of TMAO promoted gallstone formation in AKR/J mice

Lastly, we fed the AKR/J mice with LD supplemented with 0.3% TMAO in drink water and found that the incidence of gallstone formation climbed up to 70% (7/10) in these mice, while those fed with LD-only diet remained resistant to gallstone formation (0%, 0/10, Fig. 6A). Plasma TMAO massively increased about 26-fold in the TMAO-supplement group of mice compared with control mice (Fig. 6B). Measuring mRNA expression of key genes for hepatic cholesterol metabolism in AKR mice, we found mRNA expression of *Abcg5/8* and *Srb1*, significantly elevated in LD + TMAO group than in LD group (Fig. 6C, $P < 0.01$). At the protein level, TMAO increased ABCG8, SRB1 and LDLR expressions (Fig. 6D and E). These findings indicated that TMAO led to alterations in genes involving hepatic cholesterol uptake and secretion, favoring gallstone formation.

4. Discussion

In this report, we found a strong positive association between plasma TMAO level and gallstone disease in humans. To investigating the underlying mechanism, we found a dramatically increased of TMAO level in gallstone-susceptible C57BL/6J mice that were fed a LD but the TMAO level remained low in gallstone-resistant AKR/J mice. We then found increased hepatic Abcg5 and g8 expression in mice fed choline or TMAO. When feeding AKR/J mice a LD supplemented with TMAO, gallstones were induced in 70% of the mice, accompanied by an increase in hepatic Abcg5/g8 and Srb1 expression. Regulation of genes involving in cholesterol transportation by TMAO was also shown in hepatocytes.

4.1. TMAO is an environmental/dietary regulator of gallstone incidence

Genetic predisposition plays an important role in the pathogenesis of gallstone formation [32]. A Swedish twin study demonstrated that genetic factors account for a 25% in contribution to gallstone formation [33]. Since the first recognition of *Lith1* gene in mice [34], more *Lith* loci have been subsequently discovered by Paigen and Carey's group [17]. Phenotypic differences have been characterized using gallstone-susceptible and -resistant mice [35]. In this study, we found asynchronized regulation of FMO3–TMAO production between gallstone-

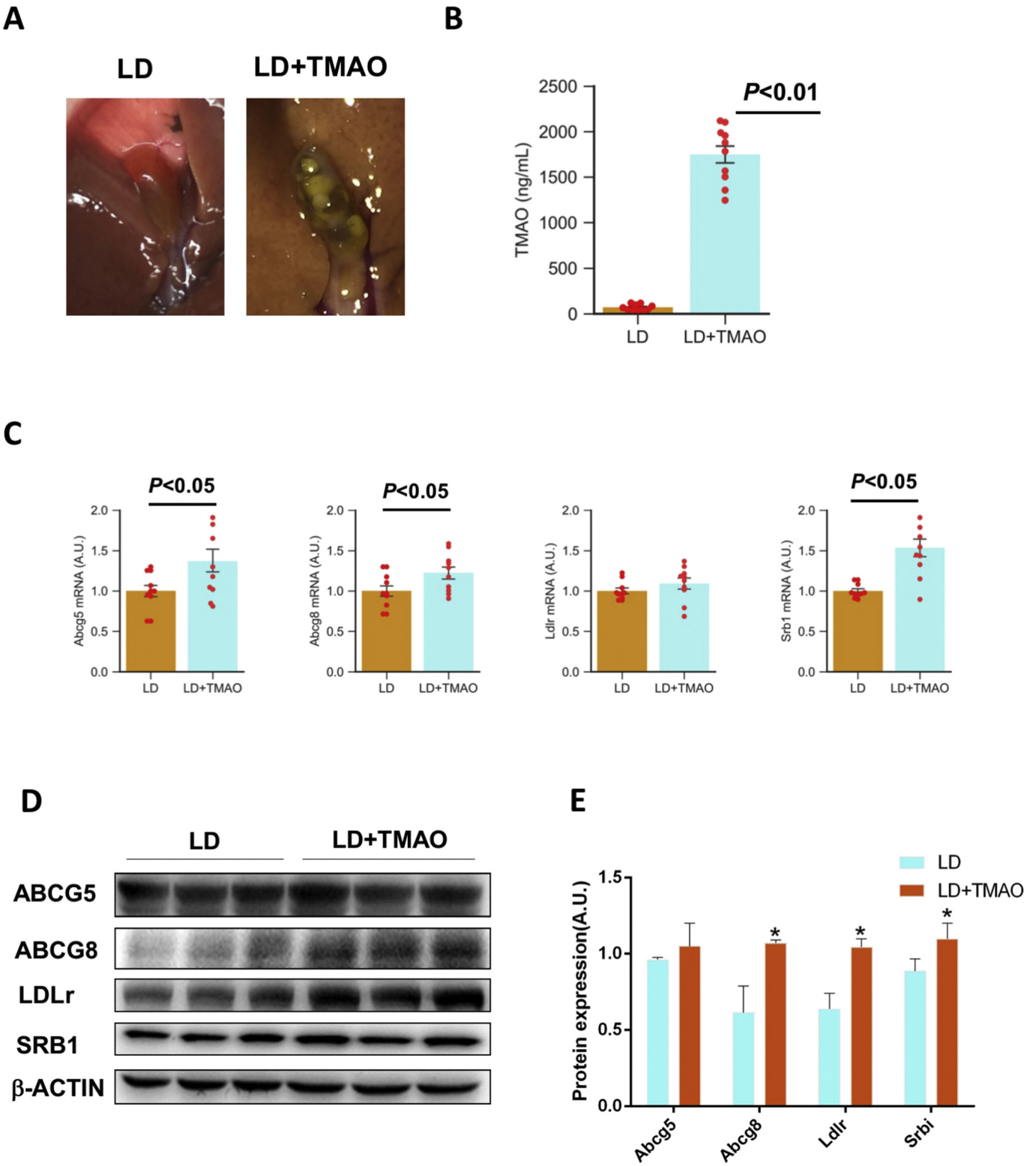


Fig. 6. Influences on gallstone formation, plasma TMAO levels, and hepatic gene expression in AKR/J mice fed a lithogenic diet + 0.3% TMAO. (A) Occurrence of gallstones in AKR/J mice fed a lithogenic diet (LD) + 0.3% TMAO in the drinking water. (B) Plasma TMAO levels; (C) Changes in mRNA expression of genes involved in hepatic cholesterol metabolism; and (D and E) Changes in protein levels involved in hepatic cholesterol metabolism in AKR/J mice fed with different diet. Data are expressed as the mean ± SEM. '*' represents $P < 0.05$; and '**' represents $P < 0.01$.

susceptible C57BL/6J mice and gallstone-resistant AKR/J mice. Gregory et al. also found differences in plasma TMAO levels between 22 strains of mice, which was strongly correlated with atherosclerotic lesion areas in aorta [36]. The phenotype could be transmitted to resistant NZW/LacJ mice by introducing TMAO-producing microbiota from atherosclerosis-prone mouse donors [36]. In this study, TMAO that was directly fed to mice successfully induced gallstone formation in gallstone-resistant AKR/J mice. These results suggest that TMAO plays

a role as an important environmental/dietary regulator to modify the incidence of certain diseases under different genetic backgrounds.

4.2. TMAO alters hepatic cholesterol metabolism favoring gallstone formation

Supersaturation of biliary cholesterol is a pre-requisite for gallstone formation [37]. At the hepatocyte canalicular membrane, enhanced ABCG5 and G8 cholesterol transporter function is responsible for cholesterol excretion into bile [38] and gallstone formation [39]. Additionally, increased hepatic cholesterol uptake via the lipoprotein receptor SRB1 was observed in gallstone-susceptible C57BL/6J mice, but not in AKR/J mice [21]. We found that mice fed different doses of TMAO and its precursor choline all had increased ABCG5/G8 expression as well as hepatic secretion of cholesterol into bile. In accordance with this finding, dietary supplementation of TMAO induced gallstone formation in AKR mice through up-regulation of both SRB1 and ABCG5/G8 expression in the liver, which could promote hepatic cholesterol uptake at the basolateral membrane and secretion into bile at the canalicular membrane. Direct gene regulation involving cholesterol uptake and secretion was further documented in mouse primary hepatocytes, human liver cells L02 and human hepatoma cells HepG2. Such regulation seems to occur under cholesterol loading conditions because TMAO did not induce Abcg5/g8 expression in mice fed a chow diet when endogenous cholesterol synthesis rates were high [2]. On contrary, antisense-oligo inhibition of FMO3 decreased TMAO production and led to decreased biliary cholesterol secretion as evidenced in several mouse models [11,40], especially under a diet rich in cholesterol [10]. These data suggest an underlying TMA/FMO3/TMAO axis in regulating hepatic cholesterol metabolism and possibly via LXR in a cholesterol-dependent manner [10].

Since the discovery of TMAO, it has attracted much attention by researchers because of its association with metabolic disorders. Strong positive relationships were reported between plasma TMAO and various cardiovascular diseases including major adverse cardiovascular events [41], acute coronary syndrome [42], heart failure [43,44], early atherosclerosis [45], renal insufficiency in chronic kidney disease [46], platelet hyper-reactivity and thrombosis risks [47], and non-alcoholic fatty liver disease [48]. Here, we showed a strong positive relationship between plasma TMAO levels and gallstone disease in the Chinese population. Because of the high coincidence of cholesterol gallstone disease with cardiovascular disease and with fatty liver disease, plasma TMAO might account for a common patho-physical factor that promotes both diseases because of its triggering effect on the metabolic disarrangement in the body.

5. Conclusions

In conclusion, we showed, for the first time, that TMAO could be associated with increased hepatic Abcg5/g8 expression, biliary cholesterol hypersecretion and gallstone formation.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbadis.2019.06.016.

Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found, in online version.

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