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Research article

Effect of hepatic NPC1L1 on cholesterol gallstone disease and its mechanism

Pingfan Mo [1], Hongtan Chen [1], Xin Jiang, Fengling Hu, Fenming Zhang, Guodong Shan, Wenguo Chen, Sha Li, Guoqiang Xu [*]

Department of Gastroenterology, Zhejiang University School of Medicine, The First Affiliated Hospital, Hangzhou, Zhejiang 310003, China

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ABSTRACT

Cholesterol gallstone disease (CGD) is associated with bile cholesterol supersaturation. The Niemann-Pick C1-like 1 (NPC1L1), the inhibitory target of ezetimibe (EZE), is a critical sterol transporter of cholesterol absorption. Intestinal NPC1L1 facilitates the absorption of cholesterol, whereas hepatic NPC1L1 promotes cholesterol uptake by hepatocytes and reduces bile cholesterol supersaturation. The potential of hepatic NPC1L1 to prevent CGD has yet to be established due to its absence in the mice model. In this study, we generated mice expressing hepatic NPC1L1 using adeno-associated virus (AAV) gene delivery. The biliary cholesterol saturations and gallstone formations were explored under chow diet and lithogenic diet (LD) with or without EZE treatment. The long-term (8-week) LD-fed AAV-mNPC1L1 mice exhibited no significant differences in biliary cholesterol saturation and gallstone formation compared to WT mice. EZE effectively prevented CGD in both WT and AAV-mNPC1L1 mice. Mechanistically, prolonged LD feeding induced the degradation of hepatic NPC1L1, whereas short-term (2-week) LD feeding preserved the expression of hepatic NPC1L1. In conclusion, our findings suggest that hepatic NPC1L1 is unable to prevent CGD, whereas EZE functions as an efficient bile cholesterol desaturator during CGD development.

1. Introduction

Cholesterol gallstone disease (CGD) is a prevalent biliary disease, afflicting about 8% of the Chinese population [1,2]. CGD patients may develop biliary complications such as biliary tract cancer, aggravating the economic and healthcare burden on patients [3]. However, CGD patients with clinical symptoms necessitate cholecystectomy treatment, whereas effective medication prophylaxis strategies are still absent [4]. Therefore, defining the underlying pathogenic mechanisms is crucial to provide potential preventive targets for CGD.

Bile cholesterol supersaturation, which is associated with elevated liver and plasma cholesterol contents, is the major pathogenic factor in CGD [5,6]. In addition, CGD arises from several pathogenic factors, including gallbladder motor dysfunction, nucleation time shortening driven by mucin hypersecretion, and gallbladder inflammation [7–9]. Niemann-Pick C1-like 1 (NPC1L1), a cholesterol transporter that is selectively expressed in the intestine of wild-type (WT) mice, serves as the target of ezetimibe (EZE) [10–12]. EZE

* Corresponding author. Department of Gastroenterology, the First Affiliated Hospital, College of Medicine, Zhejiang University, 79 Qingchun Road, Hangzhou 310006, Zhejiang, China.
E-mail address: 1193065@zju.edu.cn (G. Xu).
[1] These authors contributed equally.

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has been found to prevent CGD in wild-type (WT) mice by inhibiting the intestinal function of NPC1L1, thereby reducing the absorption of cholesterol [12–15]. However, unlike WT mice, humans express high levels of NPC1L1 in both the liver and intestine [16, 17]. Temel et al. produced transgenic mice expressing hepatic NPC1L1 (L1-Tg) to elucidate hepatic NPC1L1 function. Although bile cholesterol concentrations were reduced in L1-Tg mice, the authors believed that EZE could prevent gallstones by inhibiting dietary cholesterol absorption in the intestine, thereby reducing the cholesterol available for bile secretion [16]. However, this study did not investigate hepatic NPC1L1 function under lithogenic diet (LD) conditions, which can cause systemic lipid abnormalities [18]. Moreover, in LDLR-deficient mice fed a LD, cholesterol accumulation was observed in both the bile and liver, suggesting that the remodeling of hepatic cholesterol may increase the risk of CGD [19]. Therefore, whether the EZE can inhibit hepatic NPC1L1 function and subsequently increase the risk of CGD requires further investigation.

To assess the effect of human hepatic NPC1L1 protein expression and EZE treatment on cholelithiasis, we drove mice to express murine hepatic NPC1L1 by AAV gene delivery in this study. Compared with WT mice, the AAV-mNPC1L1 mice fed a 2-week LD exhibited decreased bile cholesterol concentrations, whereas the AAV-mNPC1L1 mice fed an 8-week LD demonstrated no significant difference, which was attributed to the degradation of hepatic NPC1L1 during prolonged LD feeding. Meanwhile, EZE could prevent CGD in both WT and AAV-mNPC1L1 mice. We conclude that hepatic NPC1L1 is unable to prevent CGD development because of the degradation of hepatic NPC1L1 during long-term LD feeding. Thus, EZE can operate as a bile cholesterol desaturator and effectively prevent CGD.

2. Materials and methods

2.1. Animals

All animal experiments were approved by the Animal Committee of Zhejiang University. The male C57BL/6 mice of 3 months old were all obtained from the Zhejiang Academy of Medical Sciences. Mice were housed in an animal facility with pathogen-free, conditions of lighting (12-h dark/light cycle), temperature (22 °C), and humidity (55 ± 5%), and were provided with an unlimited diet and water. Experiments were conducted in accordance with the ARRIVE guidelines and the National Institutes of Health guide.

2.2. Creation of liver-specific NPC1L1 expression mice model

We modulated liver-specific gene expression by AAV-mediated gene transfer method [20]. The murine NPC1L1 cDNA sequences from GenBank (No. AY437866) were synthesized by Shanghai Gene Chem Co., Ltd. (China), packaged as AAV-2/8 virus [AAV-2 inverted terminal repeat (ITR) DNA combined with the AAV-8 capsid], under a TBG promoter (AAV-TBG-NPC1L1). AAV-TBG-green fluorescent protein (GFP) was applied as a control. The intraperitoneal injection (i.p.) and intravenous (i.v.) via tail vein of AAV-TBG-GFP or AAV-TBG-NPC1L1 were at a dose of 1×10^{11} viral genomes/mouse. The intestine, liver, adipose and pancreas tissues from mice were stored in −80 °C liquid nitrogen.

2.3. Diet and drug treatment

Mice were fed a chow diet (TD.2918) or a lithogenic diet (LD, TD.90221) from Harlan Teklad Custom Research (Livermore, CA) (Supplementary Table 1). For drug treatment, mice were gavaged daily with 0.3 mg EZE suspended in 100 μl 0.4% methylcellulose or 100 μl 0.4% methylcellulose alone (VEH) as a control, commencing 1 week in advance of feeding and continuing until the end of the experiment.

2.4. Western blot

The tissues were lysed using the NE-PER™ reagents (ThermoFisher, #78833, Waltham, MA). The lysate proteins were then blotted onto the nitrocellulose membranes to analyze antibodies binding. The following antibodies were applied: NPC1L1 (ThermoFisher, #PA1-16801), GFP (Abcam, #ab291), ABCB1 (ThermoFisher, #MA1-26528), ubiquitin (Abcam, #ab134954), and tubulin (Abcam, #ab7292). The band densities of WB were measured using the ImageJ software.

2.5. Immunofluorescence (IF)

For IF analysis, sections were treated as before, followed by incubation with secondary Alexa Fluor 594 antibody (Abcam, ab150080, Bristol, UK) at 22 °C for 30 min, and slides were stained using the 4',6-Diamidino-2-Phenylindole, Dihydrochloride (ThermoFisher, D1306, Waltham, MA) for 5 min. The antibodies were same as Western blot.

2.6. Measurement of fecal cholesterol excretion

Mice were singly housed after 2-week and 8-week diet feeding. The feces collected for 3 days were dried, weighed, and then smashed into a powder. The measured feces of 50 mg were put into a glass tube with an internal standard of 103 μg 5α-cholestane. The neutral lipids from saponified feces were extracted with hexane. Using gas-liquid chromatography, the neutral sterol masses were analyzed by DIAN Laboratory (Hangzhou, China). The cholesterol masses in fecal represent the sum of derivatives (coprostanol and

coprostanone), and cholesterol masses from each sample. The excretions of fecal cholesterol were presented as μmol/day/100 g body weight.

2.7. Biochemical analysis of tissues, gallbladder bile and serum

The hepatic tissues for 150 mg from each mouse were homogenized, and then the cholesterol and triglyceride contents were analyzed by DIAN Laboratory (Hangzhou, China). Mice were anesthetized using ketamine–xylazine (50 mg/kg ip and 10 mg/kg ip, respectively) for the collection of bile and serum after an 8-h fast. The phospholipid, bile acid, and cholesterol levels in the bile as well as the triglyceride and cholesterol levels in the serum of mice were analyzed by DIAN Laboratory (Hangzhou, China). The serum FGF15 concentrations were examined with the ELISA kit (LSBio, LS-F11446, WA). Cholesterol saturation index (CSI) was calculated with Carey's critical table [21].

2.8. Examination of cholesterol crystals

After 2-week and 8-week diet feeding, the mice were euthanized to collect their gallbladders. The cholesterol crystals in gallbladder bile were examined using a polarized microscope (Leica DM5000).

2.9. Quantitative real-time, reverse transcription polymerase chain reaction

The primer sequences were all listed in Supplementary Table 2. The total RNA were extracted from tissues of mice and then analyzed using qRT-PCR, with expression data normalized to the 18s RNA expression.

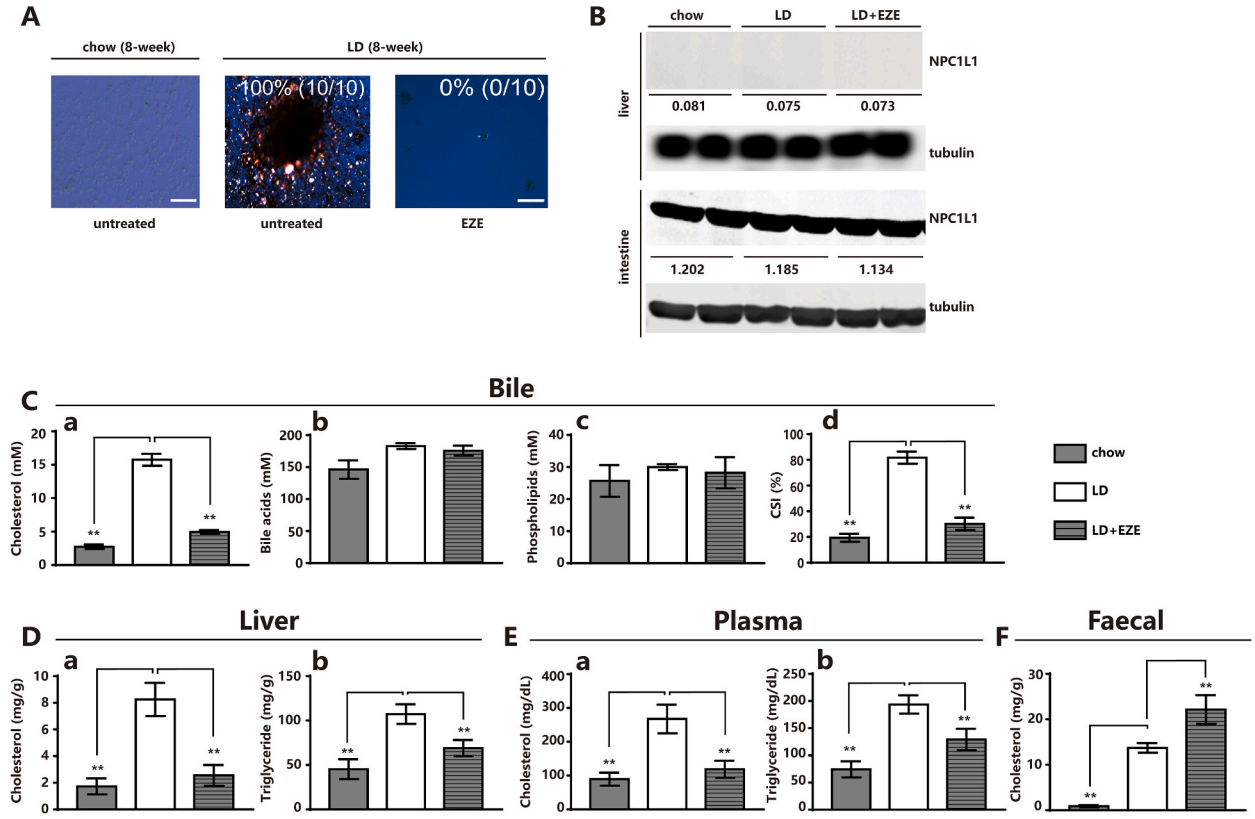

Fig. 1. Ezetimibe prevents cholesterol gallstone disease in WT mice. WT mice were fed an 8-week chow diet without ezetimibe treatment and 8-week lithogenic diet with or without ezetimibe treatment in advance of the experiments, with 10 mice per group. (A) Polarizing light microscopic examination of cholesterol crystals polymerizing into gallstones in bile. Original magnification, × 40. (B) Immunoblotting analysis was used to examine the expression of NPC1L1 in liver and intestine, with tubulin applied as controls. The immunoblotting values were measured using ImageJ software and normalized to the controls, presented below the bands. (C) Bile concentrations of cholesterol (a), bile acids (b), and phospholipid (c), (d) CSIs calculated with the data in panels a–c. (D) Liver tissue contents of cholesterol (a) and triglyceride (b), (E) Plasma contents of cholesterol (a) and triglyceride (b) from mice. (F) Cumulative feces samples collected over a 24-h period were pooled and analyzed for faecal cholesterol. Student's t-test for data with a normal distribution and Mann-Whitney *U* test for a non-normal distribution. n = 10, performed triple independently. $^{*}P < 0.05$, $^{**}P < 0.01$. WT, wide-type; LD, lithogenic diet; EZE, ezetimibe; CSI, cholesterol saturation index.

2.10. Statistics

Data were expressed with means and standard deviations (SD). Prism 8.0 was applied to perform the statistical analysis. Shapiro-Wilk test was applied to determine the normality of the data. Student's t-test was applied to compare two independent groups with a normal distribution, whereas the Mann-Whitney *U* test was applied to compare data with a non-normal distribution. $P < 0.05$ was considered significant.

3. Results

3.1. Ezetimibe prevents cholesterol gallstone disease in WT mice

To examine the preventative effect of EZE against CGD, WT mice were fed an 8-week experimental diet with or without EZE treatment before microscopic bile cholesterol crystal examination. As shown in Fig. 1A, the gallbladder bile from WT mice fed with an 8-week LD revealed larger and more frequent cholesterol crystals than those fed with a chow diet, which could be prevented by EZE treatment. As expected, WT mice did not express hepatic NPC1L1 (Fig. 1B). The LD-fed WT mice showed higher cholesterol secretion and CSIs than those fed with a chow diet, whereas the bile acids and bile phospholipids were essentially equivalent (Fig. 1C–F). Meanwhile, EZE treatment prevented the alterations in the aforementioned indices (Fig. 1C–F). In conclusion, we demonstrated that bile cholesterol supersaturation increased gallstone formation, whereas EZE effectively decreased bile cholesterol concentration and thus prevented CGD in WT mice.

3.2. Liver-specific NPC1L1 gene transfer

Given that human liver has NPC1L1, we drove mice with hepatic NPC1L1 expression to better simulate the clinical preventative effect of EZE against CGD in humans. We used AAV vectors of serotype 8 (AAV8) to express murine NPC1L1 (AAV-mNPC1L1), which are controlled by the TBG (a well-documented liver-specific promoter that restricts transgene expression to hepatocytes). Immunoblot analysis revealed that GFP expression was observed exclusively in the liver of mice injected with AAV-GFP, indicating that the infection is hepatocyte-specific. No NPC1L1 staining has been observed in the livers of mice injected with AAV-GFP (Fig. 2A). Endogenous NPC1L1 is predominantly expressed in the murine intestine, which could be recognized by NPC1L1 antibody. We observed that NPC1L1 expressions in 8-week chow diet-fed AAV-mNPC1LC mice were restricted to the intestine and liver (Fig. 2B). The expression of intestinal NPC1L1 did not change following AAV-mNPC1L1 administration (Fig. 2B). The hepatic NPC1L1 levels were comparable between 2-week and 8-week chow-fed AAV-mNPC1L1 mice, indicating its persistent expression ability (Fig. 2C). Immunofluorescence studies demonstrated that hepatic NPC1L1 was colocalized with canalicular ABCB1, indicating its hepatic canalicular location (Fig. 2D). To summarize, we successfully generated a liver-specific NPC1L1 transgenic mice model.

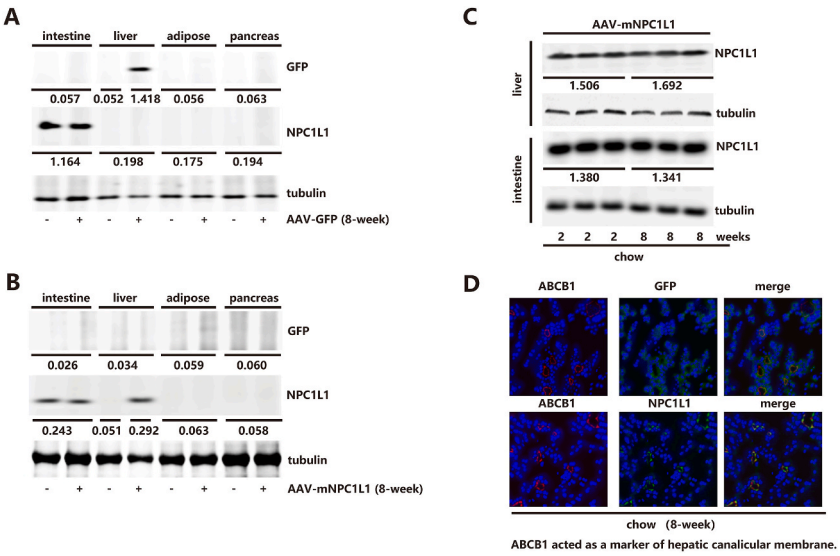

Fig. 2. Construction of liver-specific NPC1L1 expression mice. Mice injected with AAV-GFP or AAV-mNPC1L1 were fed an 8-week chow diet in advance of the experiments. Immunoblotting analysis was used to examine the expressions of (A) GFP and (B) NPC1L1 in intestine, liver, adipose and pancrea from mice, respectively. (C) Immunoblotting analysis was used to determine the expressions of NPC1L1 in liver and intestine, with tubulin applied as a control. The immunoblotting values were measured using ImageJ software and normalized to the controls, presented below the bands. (D) Confocal pictures of liver slices from mice fed 8-week chow diet that have been labeled with ABCB1, GFP and NPC1L1. The red color represents ABCB1 in hepatic canalicular membrane and green color represents GFP or NPC1L1.

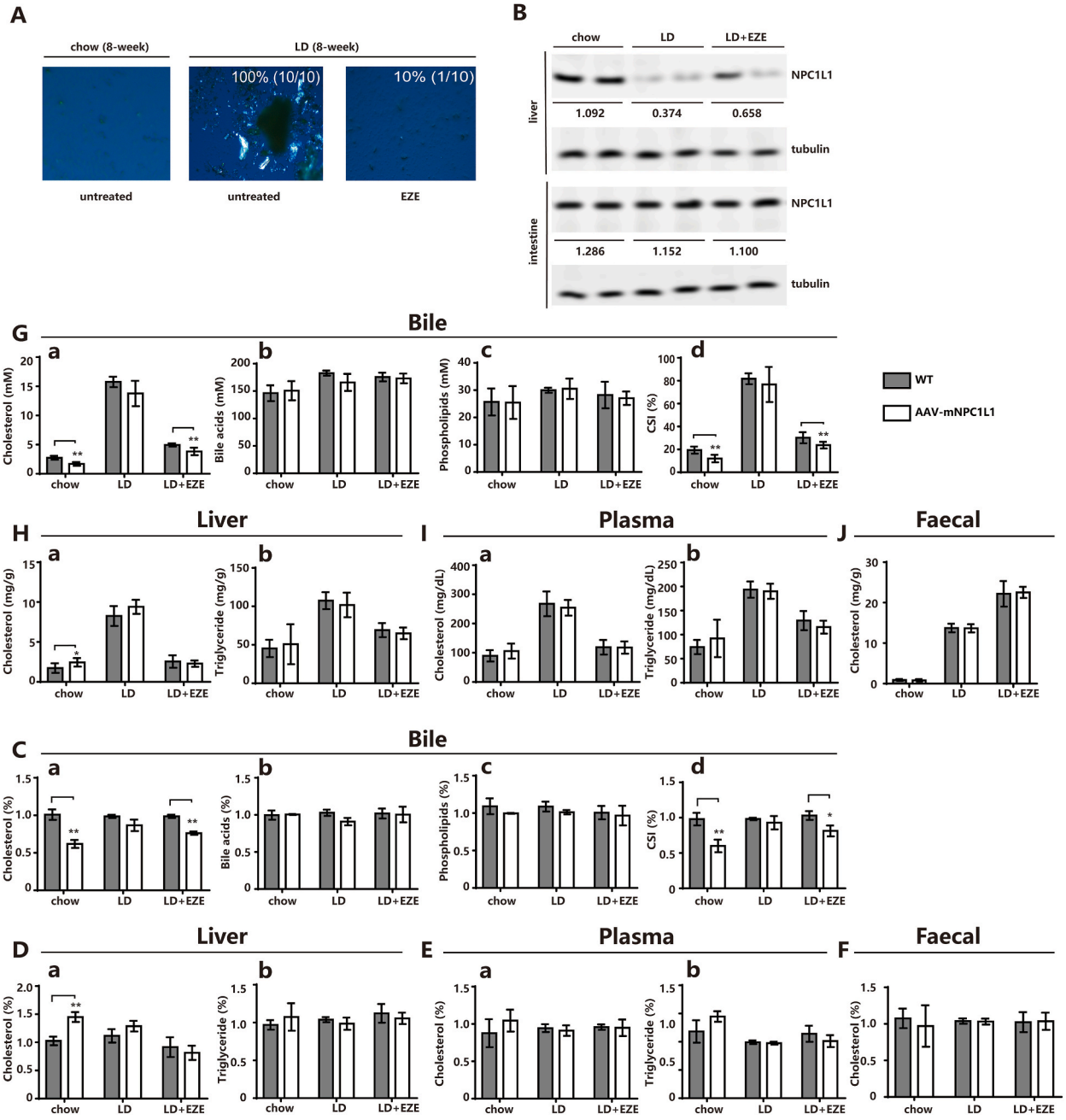

Fig. 3. Hepatic NPC1L1 had no effect on CGD development in LD-fed mice. Mice injected with or without AAV-mNPC1L1 were fed an 8-week chow and LD with or without ezetimibe treatment in advance of the experiments. (A) Polarizing light microscopic examination of cholesterol crystals polymerizing into gallstones in bile. Original magnification, × 40. (B) Immunoblotting analysis was used to examine the expressions of NPC1L1 in liver and intestine, with tubulin applied as controls. The immunoblotting values were measured using ImageJ software and normalized to the controls, presented below the bands. (C) Bile concentrations of cholesterol (a), bile acids (b), and phospholipid (c), (d) CSIs calculated with the data in panels a–c. (D) Liver tissue contents of cholesterol (a) and triglyceride (b), (E) Plasma contents of cholesterol (a) and triglyceride (b) from mice. (F) Cumulative feces samples collected over a 24-h period were pooled and analyzed for faecal cholesterol. (G) Ratios of bile concentrations cholesterol (a), bile acids (b), and phospholipid (c), (d) CSIs calculated with the data in panels a–c. (H) Ratios of liver tissue contents of cholesterol (a) and triglyceride (b), (I) Ratios of plasma contents of cholesterol (a) and triglyceride (b) from mice. (J) Ratios of cumulative feces samples collected over a 24-h period were pooled and analyzed for faecal cholesterol. Student's t-test for data with a normal distribution and Mann-Whitney *U* test for a non-normal distribution. n = 10, performed triple independently. $^{*}P < 0.05$, $^{**}P < 0.01$. WT, wide-type; LD, lithogenic diet; EZE, ezetimibe; CSI, cholesterol saturation index.

3.3. Hepatic NPC1L1 has no effect on CGD development in LD-fed mice

As expected, gallstones did not occur in chow-fed AAV-mNPC1LC mice. However, we discovered that AAV-mNPC1L1 treatment was unable to protect 8-week LD-fed mice from CGD (Fig. 3A). Meanwhile, EZE exhibited a significant preventative effect against CGD in AAV-mNPC1L1 mice, affirming its clinical prospects (Fig. 3A). We further discovered that hepatic NPC1L1 were reduced in AAV-mNPC1L1 mice fed an 8-week LD (Fig. 3B). As compared to WT mice, 8-week chow-fed AAV-mNPC1LC mice had decreased bile cholesterol concentrations and CSIs (Fig. 3C–F). However, when fed an 8-week LD, the bile cholesterol concentrations and CSIs in AAV-

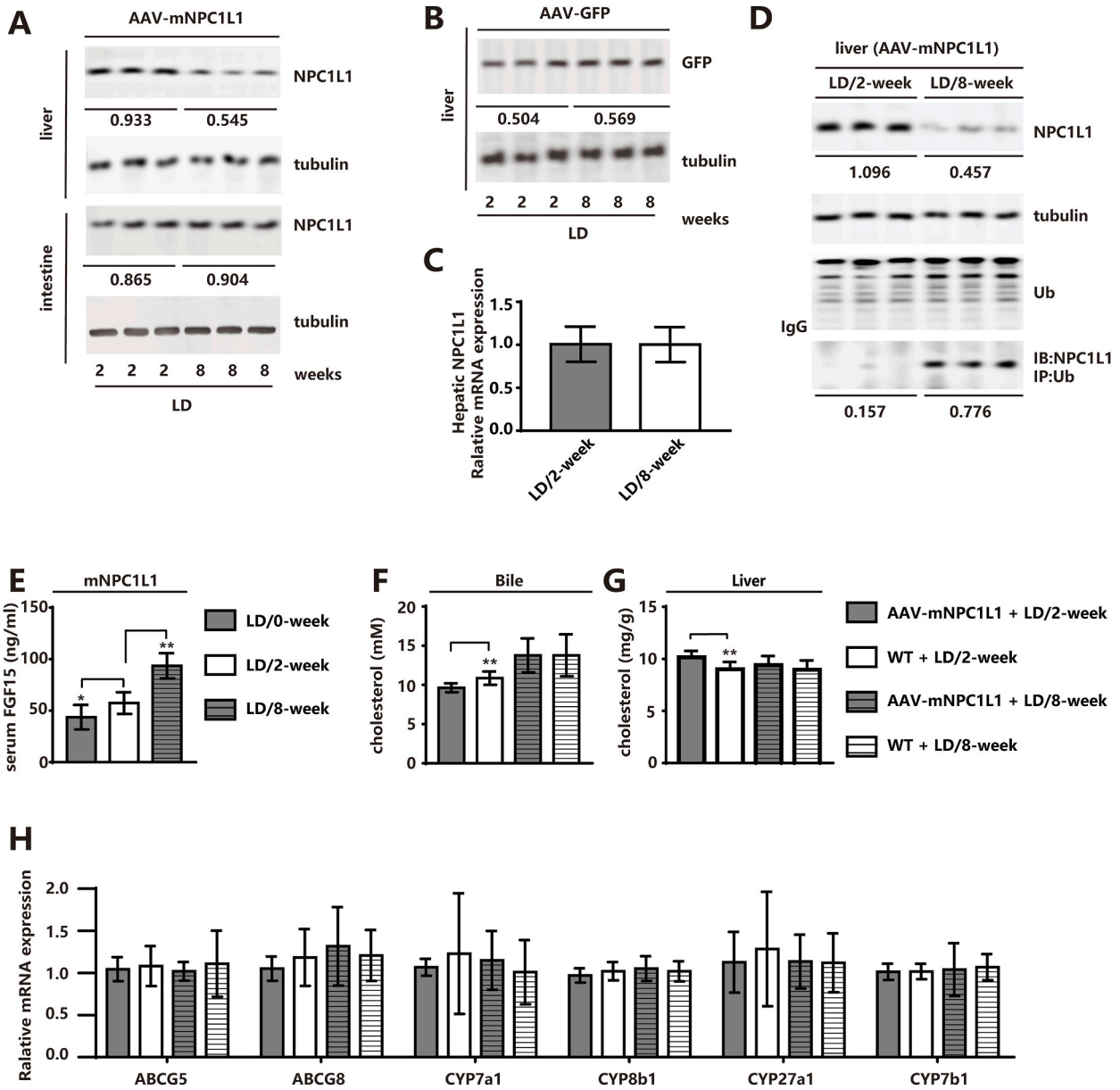

Fig. 4. Hepatic NPC1L1 decreased in long-term LD-fed mice. Immunoblotting analysis was used to examine the expressions of (A) NPC1L1 in liver and intestine from AAV-mNPC1L1 mice fed with LD for 2-week and 8-week, and (B) GFP in liver from AAV-GFP mice fed with LD for 2-week and 8-week, with tubulin applied as controls. The immunoblotting values were measured using ImageJ software and normalized to the controls, presented below the bands. (C) qRT-PCR was applied to examine the mRNA expressions of hepatic NPC1L1 from 2-week and 8-week LD-fed AAV-mNPC1L1 mice. (D) Co-immunoprecipitation result showing direct association between NPC1L1 and ubiquitination, with IgG used as controls. (E) The serum FGF15 concentrations in 0-week, 2-week and 8-week LD-fed mNPC1L1 mice. (F) Bile cholesterol concentrations and (G) liver cholesterol contents from WT and AAV-mNPC1L1 mice. (H) The hepatic mRNA expressions were analyzed by quantitative real-time PCR. Student's t-test for data with a normal distributions and Mann-Whitney *U* test for a non-normal distribution. n = 10, performed triple independently.*$P < 0.05$, **$P < 0.01$. WT, wide-type; LD, lithogenic diet; Ub, ubiquitination.

mNPC1L1 mice were comparable to WT mice (Fig. 3C–F). Interestingly, when treated with EZE, the mNPC1L1 mice fed an 8-week LD showed decreased bile cholesterol concentrations than WT mice (Fig. 3C–F). This may be due to the fact that EZE treatment reduced lipid metabolism disorders and therefore restored hepatic NPC1L1 function. Meanwhile, we examined the lipid content ratios in WT and AAV-mNPC1L1 mice and found similar results (Fig. 3G–J). Overall, we hypothesized that the impairment of hepatic NPC1L1 function may be associated with long-term (8-week) LD feeding.

3.4. Hepatic NPC1L1 protein turnover in long-term LD-fed mice

We further explored the effect of LD feeding on hepatic NPC1L1 protein levels. Our results showed that intestinal NPC1L1 expressions were similar between chow-fed and LD-fed AAV-mNPC1L1 mice (Fig. 4A). Although hepatic NPC1L1 expression showed no difference between 2-week and 8-week chow-fed AAV-mNPC1L1 mice (Fig. 2C), the 8-week (long-term) LD-fed AAV-mNPC1L1 mice showed decreased hepatic NPC1L1 protein levels than those fed with 2-week LD (Fig. 4A), whereas the GFP expressions were comparable (Fig. 4B), indicating that LD was unable to affect the long-term delivery efficacy of AAV-gene method. The AAV-mNPC1L1 mice also maintained stable mRNA expression of hepatic NPC1L1 during the LD feeding process (Fig. 4C). However, after long-term LD feeding, ubiquitination of hepatic NPC1L1 was presented in AAV-mNPC1L1 mice (Fig. 4D). Given that FGF15 can promote the degradation of hepatic NPC1L1, we further examined the serum FGF15 concentrations during different time points of LD feeding. The serum FGF15 concentrations of mNPC1L1 mice increased as LD feeding duration extending (Fig. 4E). Meanwhile, bile cholesterol concentrations and liver cholesterol contents were likewise decreased or increased in 2-week LD-fed AAV-mNPC1L1 mice, while no difference was observed in 8-week LD-fed AAV-mNPC1L1 mice, compared with WT mice (Fig. 4F and G). These findings indicated that long-term LD feeding induced the turnover of hepatic NPC1L1 protein, which was responsible for the decreased bile cholesterol absorption efficiency. Additionally, the expression of the canalicular cholesterol transporters (ABCG5/G8) and the cytochrome enzymes (CYP7a1, 8b1, 27a1, and 7b1) were comparable between 2-week and 8-week LD-fed mNPC1L1 mice (Fig. 4H), indicating that long-term LD-induced hepatic NPC1L1 degradation was the sole contributor to the difference in bile cholesterol reabsorption.

4. Discussion

The role of intestinal NPC1L1 in efficient cholesterol absorption has been well demonstrated [10]. Decreased expression of intestinal NPC1L1 can reduce plasma cholesterol levels, thereby reducing the risk of CGD in mice [22]. Conversely, hepatic NPC1L1 can prevent CGD through the down-regulation of the NPC2 protein, which facilitates cholesterol nucleation [23,24]. In humans, various NPC1L1 mutations were discovered to increase biliary cholesterol concentrations and the risk of CGD [25,26]. Since humans are more susceptible to gallstones than rodents [27], the abundance of hepatic NPC1L1 protein in humans is believed to be a reasonable defense against CGD.

Since WT mice lack hepatic NPC1L1 expression, EZE is believed to protect mice from bile cholesterol supersaturation by reducing intestinal cholesterol absorption [12–14]. Moreover, EZE can counteract the negative effects of cholesterol-supersaturated bile on gallbladder motility [15,28], making it an effective agent for preventing cholesterol gallstones [29,30]. However, human liver has NPC1L1, which reduces biliary cholesterol excretion [16,17]. In theory, EZE's inhibition of hepatic NPC1L1 function in humans may increase the bile cholesterol concentration and the risk of CGD [31,32], making it controversial whether EZE can be safely used to prevent CGD clinically.

Our previous study demonstrated that hepatic NPC1L1 degradation occurred in 8-week LD-fed mice, although the other studies did not demonstrate this phenomenon in L1-Tg mice fed a 2-week LD [33,34]. Furthermore, we also proved that this degradation was not drived from AAV delivery efficacy, and we hypothesis that it is a result of long-term stimulation, which is similar to the formation of CGD in C57BL/6 mice [35].

Given that MRP2 and BSEP could participate in the process of gallstone formation [36], we applied ABCB1 to verify the hepatic canalicular location of hepatic NPC1L1. Despite the comparable mRNA expression levels, the hepatic NPC1L1 function is impaired by long-term (8-week) but not short-term (2-week) LD feeding, which suggested that cholic acid-mediated FGF15-FGFR4 axis-induced hepatic NPC1L1 degradation may be a chronic and repeated stimulation process. Given that inhibiting the reabsorption of bile cholesterol can prevent excessive cholesterol accumulation in hepatocytes under persistently high cholesterol conditions, and that FGF15 is secreted rhythmically after meals [37], it is possible that hepatic NPC1L1 in humans is regulated dynamically by the FGF15/19-FGFR4 axis induced with cholic acid.

Humans and rodents have distinct bile acid compositions, and human bile acids are more hydrophobic [38–40]. Therefore, cholic acid compositions in LD may better simulate the hydrophobic bile acid environment of humans. Our result demonstrated that EZE reduced bile cholesterol concentrations and prevented gallstone formation in both AAV-mNPC1L1 and WT mice fed with LD, indicating its potential preventive effect on CGD. This result is consistent with the instruction of EZE, which increased bile cholesterol levels by 2–4 fold after one month in dogs that express NPC1L1 in both the intestine and liver, did not cause gallstone formation after one year [41]. Ezetimibe blocks the internalization and retention of intestinal NPC1L1, which explains our result that EZE treatment did not affect the intestine NPC1L1 protein levels [42]. Furthermore, mNPC1L1 mice treated with EZE and fed with LD showed a decrease in bile cholesterol concentrations compared to WT mice, possibly due to the restoration of hepatic NPC1L1 function through the reduction of lipid metabolism disorders. Therefore, it is conceivable that EZE treatment could restore hepatic NPC1L1 function, to some extent, providing further evidence of its preventive effect on CGD.

It was demonstrated that the FGF15-FGFR4 axis induced by cholic acid promotes the ubiquitination of hepatic NPC1L1 [20]. Meanwhile, FGF15 released by the intestine inhibits bile acid synthesis in the liver by reducing Cyp7a1 transcription through FGFR4

and SHP [37,43,44]. Additionally, the FGF15-FGFR4-SHP axis regulates cholesterol transport genes, including Acat2, the *trans*--acyltransferase for cholesterol esterification [45,46]. Thus, FGF15 appears to exert a variety of effects on bile cholesterol metabolism and gallstone formation. Given that the mice fed with LD more closely simulate the hydrophobic bile canalicular environment in humans than those fed with chow diet, the function of hepatic NPC1L1 in humans still remains to be determined. The possible explanation is that hepatic NPC1L1 is associated with greater bile detergent properties to the lipid bilayer plasma membrane of human hepatocytes, which requires further investigation.

In this study, we discovered that prolonged feeding on LD resulted in the degradation of hepatic NPC1L1, which impaired its ability to facilitate bile cholesterol reabsorption. Meanwhile, EZE treatment significantly reduced the risk of CGD, which dispelled concerns about EZE's potential harmful effects on hepatic NPC1L1 function and subsequent long-term CGD prophylaxis.

5. Conclusions

Long-term LD induces hepatic NPC1L1 ubiquitination and degradation, thereby abrogating its prevention of cholesterol gallstone disease. Ezetimibe, an NPC1L1 inhibitor, can prevent CGD by inhibiting exclusively intestinal NPC1L1 but not hepatic NPC1L1.

Declarations

Author contribution statement

Pingfan Mo, Hongtan Chen: Performed the experiments; Wrote the paper.

Xin Jiang, Sha Li: Analyzed and interpreted the data.

Fengling Hu, Fenming Zhang, Guodong Shan, Wenguo Chen: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Guoqiang Xu: Conceived and designed the experiments.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

No potential conflict of interest was reported by the authors.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e15757.

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