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RESEARCH COMMUNICATION

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Causal effects from nonalcoholic fatty liver disease on cholelithiasis: A mendelian randomization study

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Abstract

Background and Aims: Both nonalcoholic fatty liver disease (NAFLD) and cholelithiasis are highly prevalent hepatobiliary diseases with risk of progression into severe outcomes. Considering the close relationship between liver and gallbladder in anatomy and physiology, a potential causal relationship between NAFLD and cholelithiasis has been speculated.

Methods: Mendelian randomization (MR) was employed using genome-wide association study (GWAS) summary statistics in Million Veteran Program (MVP) for NAFLD, and statistics in UK biobank for cholelithiasis.

Results: Our results demonstrate that NAFLD has a causal effect on cholelithiasis risk (OR, 1.003; 95%CI, 1.000–1.006; $p = 0.03$). We also performed the sensitivity analysis and heterogeneity test to ensure the accuracy of outcome and avoid the reverse causality.

Conclusion: NAFLD should be regarded as a potential pathogenic factor in pathogenesis study of cholelithiasis, and be considered in assessment and treatment of cholelithiasis.

KEYWORDS
causality, cholelithiasis, mendelian randomization, nonalcoholic fatty liver disease

1 | INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD), a common hepatobiliary disease, has become the most common cause of chronic liver disease worldwide. The global prevalence of NAFLD is rapidly increasing, with an estimated number of 30% at present.[1] It is regarded as the hepatic manifestation of metabolic syndrome.[2] As a chronic liver disease, NAFLD per se can progress into fibrosis, cirrhosis, and liver cancer.[3] But more importantly, it can also increase the risk of various diseases, including type 2 diabetes, atherosclerosis, cardiovascular disease, and chronic kidney disease. Therefore, there is a need to further clarify the interaction between NAFLD and other diseases.

Cholelithiasis is another common hepatobiliary disease, jeopardizing 10–20% of adults worldwide.[4] It can lead to severe complications such as cholecystitis, cholangitis, and pancreatitis.[5] The risk factors of cholelithiasis partially overlap with the risk factors of NAFLD, including insulin resistance, obesity, diabetes mellitus and hyperlipidemia.[5,6] Thus, it is likely to occur simultaneously in many patients with partial overlap in pathogenesis. From the perspective of pathophysiology, it has been proposed that NAFLD can lead to cholesterol hyper-saturation and

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nucleation in bile, which are considered the main promoters of nucleation. Low bile salt levels and impaired gallbladder movement caused by NAFLD are also thought to be causative factors for cholelithiasis.[7] In accordance with this pathophysiological hypothesis, previous observational studies have shown that patients with NAFLD have an increased risk of cholelithiasis.[8,9] However, some other observational studies reveal no correlation between them.[10,11] The discrepancy in these studies may stem from inevitable confounding bias in traditional observational studies. Therefore, whether there is a causal relationship between NAFLD and cholelithiasis remains unknown.

Mendelian randomization (MR),[12] a method to explore causality, can effectively avoid the above limitations by using genetic variants as a tool for exposure. Genetic associations are unaffected by confounding or reverse causation, as genes are randomly allocated at conception. In this study, we performed a two-sample MR analysis to investigate the causal association of NAFLD with cholelithiasis.

2 | METHODS

2.1 | Study design

We used two-sample MR approach to detect the causal relationship between NAFLD and cholelithiasis. The MR method uses single nucleotide polymorphisms (SNPs) as instrumental variables (IVs). The study design is shown in Fig. 1. There are three key assumptions that underlie MR[13]: (1) a significant association exists between genetic variants and exposure, (2) the IVs should be independent of potential confounders associated with exposure factors and outcomes, (3) the IVs should affect the outcomes only through the exposure. The summary statistics of NAFLD was obtained from the GWAS study published by Vujkovic et al.[14] In this study, NAFLD was defined as chronically elevated alanine transaminase (cALT) levels after exclusion of other liver diseases. This study involved 68725 European NAFLD cases and 95472 controls. The summary statistics associated with cholelithiasis was obtained from the IEU with a GWAS ID as "ukb-a-559". The cholelithiasis was defined by codes of the International Classification of Diseases 10th Revision (ICD-10), Which involved 6986 cholelithiasis cases and 330213 controls.

2.2 | Selection of instrumental variants

The strict criteria were used to select qualified IVs. 55 SNPs strongly associated with NAFLD in European ancestry were extracted as candidate IVs ($P < 5E\text{-}08$) (Supplementary Table 1). Then 20 SNPs were removed based on the linkage disequilibrium (LD) clumping ($r^2 < 0.001$ within a 10,000 kb range), and all the resting SNPs were available in the outcome, therefore no proxy SNP was used. Afterwards, the F statistics were calculated to present the strength between IVs and NAFLD. IVs with an F statistic > 10 were considered as valid and reliable IVs, and no SNP was removed in this step. We then queried these SNPs in PhenoScanner database to determine their association with potentially confounding phenotypes that could lead to horizontal pleiotropy. The confounding phenotypes include dyslipidemia-related phenotypes (low-density lipoprotein (LDL), triglycerides and total cholesterol), obesity-related phenotypes (body mass index (BMI), waist circumference, hip circumference) and outcome-related phenotype (cholelithiasis), and 16 SNPs were removed in this step. Finally, we conducted a series of sensitivity analysis including MR-PRESSO, Cochrane's Q test, MR Egger regression, and Steiger-test filtering to exclude SNPs that affect result stability, and 5 SNPs were excluded in this step.

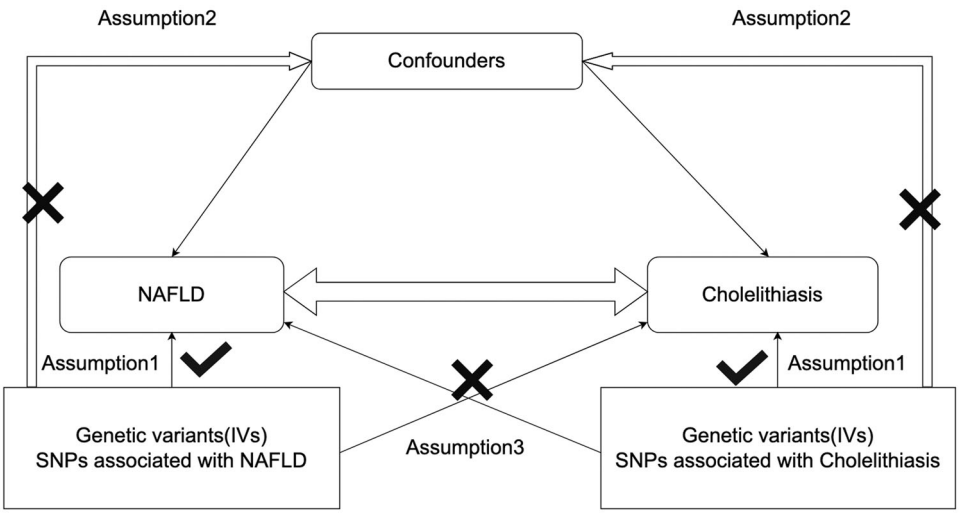

FIGURE 1 A directed acyclic graph of MR model. Assumption 1: IVs are strongly correlated with exposure. Assumption 2: IVs are not related to confounding factors. Assumption 3: IVs are independent of outcomes (i.e., IVs can only affect outcomes through exposure). MR, Mendelian randomization; IVs, instrumental variables.

2.3 | Statistical analysis

MR approaches including inverse-variance weighted (IVW) model, weighted median, Weighted mode, MR-Egger, Simple mode and MR-RAPS method were used to determine MR estimates between NAFLD and cholelithiasis. We applied IVW estimates for the main analysis. R^2 represented the exposure's genetic variance explained by IVs. F-statistics (>10) were used for evaluation of the strength of the IVs. We also performed the sensitivity analysis and heterogeneity test to detect underlying pleiotropy and heterogeneity. The Cochrane's Q value was calculated to evaluate heterogeneity. Outliers detected by MR Egger regression and MR-PRESSO were removed to reduce horizontal pleiotropy and correct the IVW estimation. The leave-one-out sensitivity analysis was performed to test the robustness of this study. All statistical analyses were undertaken using the TwoSampleMR and mr.raps packages in R statistical software version 4.2.0. A two-tailed $p < 0.05$ was considered statistically significant.

3 | RESULTS

In our MR analysis, none of the SNPs was weak instrumental variable (F statistics < 10). After removing outliers due to horizontal pleiotropic and ambiguous palindrome, and excluding SNPs was associated with potential confounding, we finally included 14 SNPs as IVs (Supplementary Table 2). In the IVW analysis, NAFLD was positively associated with cholelithiasis (OR, 1.003; 95%CI, 1.000–1.006; $p = 0.03$) (Fig. 2). Similar results were also observed in other analysis methods including Weighted median (OR, 1.004; 95%CI, 1.000–1.008, $p = 0.07$), Weighted mode (OR, 1.004; 95%CI, 1.000–1.009, $p = 0.09$), MR Egger (OR, 1.004; 95%CI, 0.997–1.010, $p = 0.28$), Simple mode (OR, 1.000; 95%CI, 0.992–1.008, $p = 0.96$) and MR-RAPS (OR, 1.003; 95%CI, 1.000–1.006, $p = 0.03$) (Fig. 2). There was no evidence of heterogeneity (Q = 11.832, $p = 0.54$) or pleiotropy (egger intercept = −0.00005, $p = 0.84$). In sensitivity analysis, we performed Steiger filtering to detect the directionality of single SNPs to avoid reverse bias, and the results showed that all SNPs were in the correct direction. The leave-one-out sensitivity analysis showed that the association between NAFLD and cholelithiasis was not substantially driven by any individual SNP (Supplementary Fig. 1). And the funnel plot indicated no significant asymmetry of the MR analysis (Supplementary Fig. 2). The contributions of individual SNPs and overall estimated effects are shown in Supplementary Fig. 3.

4 | DISCUSSION

We performed a two-sample MR analysis based on publicly available summary-level data to investigate the causal relationship between NAFLD and cholelithiasis. In our study, we found that NAFLD has a causal effect on cholelithiasis risk.

Till now, the relation between NAFLD and cholelithiasis remains unclear. Some observational studies reveal a positive association between NAFLD and cholelithiasis.[8,9] A large prospective cohort study in Korea revealed that the presence and severity of NAFLD were in positive association with the incidence of cholelithiasis, while the presence of cholelithiasis was also positively associated with NAFLD incidence.[15] In addition, a case-control study in Slovakia identified cholelithiasis as an independent risk factor for NAFLD, and vice versa.[16] A cross-sectional study in Italy also found that patients with biopsy-proven NAFLD had a higher incidence of cholelithiasis than ordinary people, and they also found that cholelithiasis was positively associated with NAFLD severity.[17] However, other studies have shown no association between NAFLD and cholelithiasis. After adjusting for risk factors linked to NAFLD and cholelithiasis, an epidemiological survey conducted in the United States revealed no discernible correlation between the initiation of NAFLD and cholelithiasis.[11] Another cross-sectional study conducted in Korea revealed no significant association between cholelithiasis and the onset of NAFLD.[10] The discrepancy in these studies may be caused by study biases from unavoidable limitations in observational studies. For example, the onset of both NAFLD and cholelithiasis are influenced by shared pathological factors such as insulin resistance, constituting a potential confounding bias. Observational studies per se lack randomization and blinding, leading to potential influence of unknown confounding bias. In addition, different diagnostic methods employed by different studies may contribute to different efficacy in NAFLD identification and evaluation.

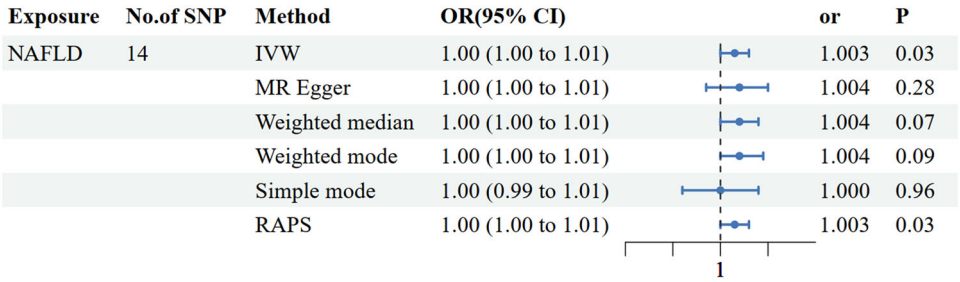

FIGURE 2 Mendelian randomization analysis results for nonalcoholic fatty liver diseases (NAFLD) and cholelithiasis; OR, the odds ratio. 95% CI, 95% confidence interval; SNP, single-nucleotide polymorphism.

The main pathogenesis mechanism of cholelithiasis basically involves dysregulations in hepatic cholesterol (e.g. apolipoprotein genetic variant-associated increase in cholesterol secretion) and bilirubin metabolism (e.g. UGT1A1 genetic variant-associated hyperbilirubinemia[18–20]), which lead to cholesterol and/or bilirubin supersaturation and consequent crystal precipitation in bile acid. Our results show the causal effect of NAFLD on cholelithiasis risk, which is consistent with the positive correlation between these two diseases found in previous observational studies. However, it remains unclear why NAFLD can influence the pathogenesis of cholelithiasis. One possible mechanism involves farnesoid x receptor (FXR)-related dysregulation in bile acid formation. As a bile acid nuclear receptor with high expression in liver, FXR plays an indispensable regulatory role in bile formation by sustaining the expression of ABCB11 and ABCB4, two canalicular transporters responsible for transporting bile acids and phosphatidylcholine into bile and consequently sustaining the solubility of biliary cholesterol. However, NAFLD can lead to a decrease in hepatic FXR expression,[21] resulting in a decrease in hepatic ABCB11 and ABCB4 expression and a consequent decrease in bile cholesterol solubility. Correspondingly, a previous basic study showed that FXR-ablated mice were more prone to form gallstones when fed with a lithogenic diet.[22] Another possible mechanism involves aberrant activation of hypoxia-inducible factor 1α (HIF-1α). The hypoxic microenvironment of steatotic liver increases hepatic HIF-1α expression, leading to decreased AQP8 expression and consequent decreased water secretion from hepatocytes, ultimately contributing to higher biliary lipid concentration and susceptibility of cholesterol stone formation.[23]

The findings of our study are of both high reliability and high translational value. From the perspective of study reliability, large number of GWAS cases are employed here, guaranteeing a large sample size in our study. In addition, due to random arrangement of genetic variants at fertilization, MR analysis is less prone to be affected by confounding bias and reverse causation than conventional observational studies. From the perspective of translational value, patients with NAFLD should be screened for cholelithiasis under the premise of potential NAFLD-originated cholelithiasis. Furthermore, NAFLD-related cellular and molecular alterations should be tentatively considered when exploring the pathogenesis of cholelithiasis. Similarly, NAFLD-related therapies, including lifestyle changes and drug treatments, should be incorporated into the treatment of cholelithiasis when patients are comorbid with NAFLD.

Some inevitable limitations exist in our study. Firstly, all GWAS data in this study are from European populations, therefore whether the findings in this study can be applied to other populations remains to be explored. Secondly, epidemiological studies have shown that NAFLD and cholelithiasis are influenced by gender and age. However, due to a lack of sex and/or age-stratified data, we could not further evaluate the causal relationship between genetically predicted NAFLD and cholelithiasis in different genders and age groups. Thirdly, both cholelithiasis and NAFLD are heterogenic diseases, but we cannot further analyze disease subtypes due to the lack of relevant stratified data. Further studies with consideration of different cholelithiasis and/or NAFLD subgroups should be implemented in the future.

AUTHOR CONTRIBUTIONS

Yin-Shi Su: Data curation; Formal analysis; Methodology; Writing—original draft. Shuang-Zhe Lin: Data curation; Writing—review & editing. Yuan-Wen Chen: Conceptualization; Supervision; Writing—review & editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. All authors have read and approved the final version of the manuscript. Yuan-Wen Chen had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis

ETHICS APPROVAL AND PATIENT CONSENT STATEMENT

Ethical approval and informed consent had been obtained in all original studies cited in this work.

TRANSPARENCY STATEMENT

The lead author Yuan-Wen Chen affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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