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Exploring potential plasma drug targets for cholelithiasis through multiancestry Mendelian randomization

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Background: Cholelithiasis poses significant health and economic burdens, necessitating novel pharmacological targets to enhance treatment efficacy.

Method: Based on genome-wide association analysis studies, the authors performed a two-sample Mendelian randomization (MR) analysis based on plasma proteomics to explore potential drug targets in European (n_{Case} = 40 191 and $n_{Control}$ = 361 641) and Asian (n_{Case} = 9305 and $n_{Control}$ = 168 253) populations. The authors confirmed the directionality and robust correlation of the drug targets with the results through reverse MR analysis, Steiger filtering, Bayesian colocalization, phenotype scanning, and replication in multiple databases. Further exploration of the safety and possible mechanisms of action of phenome-wide MR analysis and protein–protein interactions (PPIs) as individual drug targets was performed.

Results: Our proteomics-based MR analyses suggested that FUT3 (OR = 0.87; 95% CI: 0.84–0.89; $P = 4.70 \times 10^{-32}$), NOE1 (OR = 0.58; 95% CI: 0.52–0.66; $P = 4.21 \times 10^{-23}$), UGT1A6 (OR = 0.68; 95% CI: 0.64–0.73; $P = 9.58 \times 10^{-30}$), and FKBP52 (OR = 1.75; 95% CI: 1.37–2.24; $P = 8.61 \times 10^{-6}$) were potential drug targets in Europeans, whereas KLB (OR = 1.11; 95% CI: 1.07–1.16; $P = 7.59 \times 10^{-7}$) and FGFR4 (OR = 0.94; 95% CI: 0.91–0.96; $P = 4.07 \times 10^{-6}$) were valid targets in East Asians. There was no reverse causality for these drug targets. Evidence from Bayesian colocalization analyses supported that exposure and outcome shared consistent genetic variables. Phenome-wide MR analysis suggested the potential deleterious effects of NOE1 and FGFR4. PPI analysis confirmed the pathways associated with the potential targets involved in bile acid metabolism.

Conclusions: Genetically predicted levels of the plasma proteins FUT3, NOE1, UGT1A6, and FKBP52 have the potential as prospective targets in Europeans. Moreover, the plasma levels of KLB and FGFR4 may serve as potential targets for the treatment of cholelithiasis in East Asians.

Keywords: causality, cholelithiasis, drug target, Mendelian randomization

Introduction

Cholelithiasis is a common medical condition characterized by the presence of gallstones in the gallbladder or bile ducts. Gallstones are small, hardened deposits formed from bile components such as cholesterol or bilirubin. Gallstones occur when there is an imbalance in the composition of the bile, resulting in the formation of solid particles. The exact causes of this imbalance are not fully understood, but factors such as obesity, high-fat diets, certain medications, and certain medical conditions such as liver disease or diabetes may increase the risk. For many patients, gallstones may remain asymptomatic; nonetheless, they can cause a myriad of complications that warrant emergent medical intervention. Recognizing the adverse impacts of cholelithiasis, the pursuit of more efficacious pharmaceutical interventions is crucial for diminishing the clinical and economic burden of this prevalent hepatobiliary disorder[1].

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While surgical and endoscopic interventions remain the primary management options for gallstones, the development of effective and reliable pharmacological treatments is an area of ongoing research and unmet clinical needs[2–4]. As the main type of drug target, protein targets play a key role in drug development. With advances in genomics and proteomics technology, the mechanisms and functions of many proteins have been elucidated. Mendelian randomization (MR) was developed based on Mendel's laws of heredity that usually utilizes single nucleotide polymorphisms (SNPs) from genome-wide association studies (GWAS) as genetic instruments to estimate the causal effect of an exposure on an outcome. The advantage of MR is that it utilizes an individual's genetic information and avoids the confounding and covariable biases common in conventional observational studies. Thus, MR can be used to guide drug target development and drug repurpose[5,6].

In this study, we conducted a multiancestry MR study to explore potential drug targets in European and East Asian populations. We first identified possible plasma proteins as potential targets for the treatment of cholelithiasis by MR analysis. Multiple sensitivity analyses and different validation sets were subsequently used to ensure the robustness of the results. Finally, we explored the potential side effects of the drugs as well as protein interactions.

HIGHLIGHTS

- A systematic investigation between cholelithiasis and plasma proteins was carried out through this drug-target Mendelian randomization (MR) analysis.
- There are differences between European and East Asian ethnicities regarding effective drug targets for cholelithiasis.
- In European ancestry, this MR study revealed FKBP52 as a potential risk factor for cholelithiasis, while FUT3, NOE1, and UGT1A6 have been implicated as protective factors. In East Asian ancestry, this MR study revealed KLB as a potential risk factor, while FGFR4 has been implicated as a protective factor against cholelithiasis.
- This study goes beyond traditional association studies by not only identifying these novel targets but also suggesting possible pathways through which emerging therapies could modify disease risk.

Materials and methods

Our multiancestry MR study design is shown in Figure 1. This study was performed with reference to the guidance reported by STROBE-MR[5] (Table S1, Supplemental Digital Content 1, http://links.lww.com/JS9/D30).

Plasma protein quantitative trait loci

In particular, we used genetic data at the summary level from the deCODE consortium of Ferkingstad *et al*.[7] and the UK Biobank Pharma Proteomics Project (UKB-PPP) consortium of Sun *et al*.[8] (Table S2, Supplemental Digital Content 1, http://links.lww.com/JS9/D30). Measured using the SomaScan proteomics platform (version 4; SomaLogic), the deCODE consortium encompassed a wide range of genetic associations for 4907 circulating plasma proteins in a population of 35 559 Icelandic ancestry (https://www.decode.com/summarydata/). Age and sex adjustments were made to the aptamers. In addition, the UKB-PPP consortium performed proteomic analysis of plasma samples collected from 262 East Asian participants using the antibody-based Olink Explore 3072 PEA and measured 2941 protein analytes. The final results were adjusted for age, sex, and BMI. Considering that cis-pQTLs better explain causal pathways between proteins and disease, they were used to represent the corresponding protein-associated drug targets. We first extracted all cis-pQTL data strongly correlated with the corresponding exposure ($P < 5 \times 10^{-8}$). Second, all loci in the major histocompatibility complex interval (chr6, 26-34 Mb) were removed because instrumental variables in this interval have complicated pleiotropy in drug-target studies[9]. We further removed unqualified instrumental variables in a 10 Mb window and linkage disequilibrium (LD) clumping ($r^2 < 0.001$)[10]. We calculated the proportion of variance explained and the F statistic for each genetic variant, which was considered a powerful instrumental variable when the F statistic was greater than 10[11].

Summary of the outcomes of cholelithiasis GWAS data

To avoid sample overlap while maximizing the likelihood of exploring potential drug targets, we used pooled GWAS data for cholelithiasis from the FinnGen consortium for Europe[12] and from the BioBank Japan for East Asia[13] (Table S2, Supplemental Digital Content 1, http://links.lww.com/JS9/D30). In brief, GWAS pooled data on cholelithiasis from the FinnGen consortium were obtained from the 10th round of publicly available datasets (available at the website https://finngen.gitbook.io/documentation/), which included 40 191 cases and 361 641 controls. During the analysis, corrections were made for sex, age, the first 10 principal components, and the genotyping batch. For East Asian ancestry, we extracted genetic summary data from the cholelithiasis population in the BioBank Japan, which included 9305 cases and 168 253 controls. A GWAS for cholelithiasis was performed by using a generalized linear mixed model (v.0.37) implemented in SAIGE and adjusted for the first 20 principal components, such as age and sex.

Statistical analysis

Mendelian randomization analysis

We used plasma cis-pQTLs from different ancestry sources (European and East Asian) as exposures and two independent cholelithiasis datasets as endpoints. We performed a proteome-wide two-sample MR analysis. For a specific protein, when only one cis-pQTL was available, we used the Wald ratio method for causal effect estimation. When more than one cis-pQTL was available, we used the inverse-variance weighted (IVW) method for estimation and Cochran's Q test to explore heterogeneity. A P-value of less than 1.02×10^{-5} or 1.70×10^{-5} (0.05/4907 or 0.05/2940, Bonferroni's method) was considered to indicate statistical significance and that the potential drug target had an impact on the disease. We also removed cis-pQTLs, which are strongly associated with risk factors for cholelithiasis at the potential genome-wide level of association, from the PhenoScanner database (version 2, http://www.phenoscanner.medschl.cam.ac.uk/)[9].

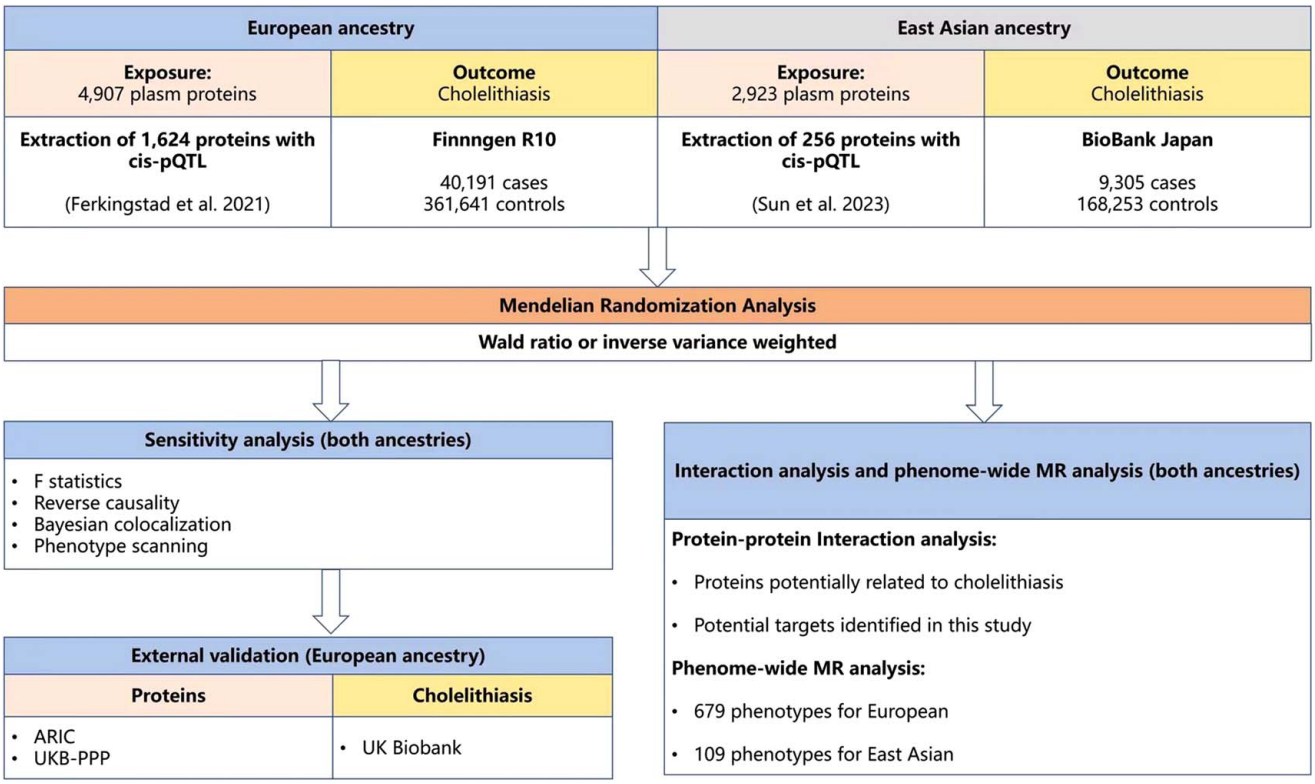

Figure 1. Study design for identifying genetically predicted circulating plasma proteins with causal effects in European and East Asian populations.

Reverse causality detection

To further ensure the directionality of our identified drug targets, we performed the Steiger filtering test and bidirectional MR[14]. A *P*-value greater than 0.05 indicated that the results were reverse confounding factors. In the reverse MR analysis, we performed a two-sample MR analysis with genetically predicted cholelithiasis as the exposure variable and identified drug target proteins as the outcome. The reverse MR analysis consisted of five methods: IVW, weighted median (WM), MR–Egger, simple, and weighted. Reverse confounding bias was considered to exist when the *P*-value calculated based on the IVW method was less than 0.05.

Bayesian colocalization analysis

Bayesian colocalization analysis was used to explore whether the same loci leading to causality existed between exposure and outcome phenotypes[15]. The results of the analysis are based on five different assumptions[16]. Specifically, we focused on the posterior probability of Hypothesis 4 (PPH_4) of Bayesian colocalization in the present study by observing that the identified drug targets share the same variables in the same region as those associated with cholelithiasis. When the PPH_4 concentration is greater than 0.8, we believe that the protein represented by the cis-pQTL and that cholelithiasis were caused by the same genetic variant. Hence, plasma protein is considered a potential drug target for the treatment of cholelithiasis.

External validation

The cis-pQTLs from the Atherosclerosis Risk in Communities (ARIC) and UKB-PPP cohorts served as the validation cohorts representing exposed protein drug targets[8,17] (Table S2, Supplemental Digital Content 1, http://links.lww.com/JS9/D30). The ARIC cohort included 7213 European Americans and reported 2004 plasma proteins, while the UKB-PPP cohort had 2941 plasma proteins measured in 54 219 individuals using an antibody-based proximity extension assay (PEA). The cis-pQTL genes from these two cohorts were considered for inclusion in the validation analysis. We also used another cholelithiasis GWAS from the UK Biobank as an outcome for validation (Table S2, Supplemental Digital Content 1, http://links.lww.com/JS9/D30). The relevant data for the UK Biobank consortium are from the publicly available MRC-IEU open GWAS (GWAS ID: ukb-b-18700), which includes 7682 cases and 455 251 controls[18]. Protein targets that were externally validated were considered promising targets for the treatment of cholelithiasis, while targets that were not validated were considered suggestive.

Phenome-wide Mendelian randomization

Finally, we explored the possible side effects of the identified drug targets aimed at treating cholelithiasis using phenome-wide MR analysis. For European ancestry, we incorporated 679 disease phenotypes with reference to the study by Chong *et al*.[19] (Table S3, Supplemental Digital Content 1, http://links.lww.com/JS9/D30). Like in the previous study, disease outcomes were based on PheCodes definitions. Because of the lack of data availability and

sufficient statistical efficacy, we excluded sex-specific related outcomes and outcomes from fewer than 500 patients. For East Asian ancestry, we accessed all available phenotypes from BioBank Japan in the MRC Integrated Epidemiology Unit (MRC-IEU). We removed duplicate phenotypes and retained the phenotype with the largest sample size. Finally, 109 East Asian ethnic phenotypes were included in the phenome-wide MR analysis (Table S4, Supplemental Digital Content 1, http://links.lww.com/JS9/D30). A *P*-value less than 7.36×10^{-5} or 4.59×10^{-4} (0.05/679 or 0.05/109, Bonferroni's method) was considered to indicate the presence of potential side effects. All of our statistical analyses were performed in R (version 4.3.1) using the 'TwoSampleMR' package (version 0.5.7) and the 'coloc' package (version 5.1.0).

Protein–protein interaction network

Considering the key role of bile acid metabolism in cholelithiasis formation, we explored the possible underlying mechanisms by identifying protein–protein interactions (PPIs) linking identified drug targets to key proteins involved in bile acid metabolism[20]. With a minimum interaction score of 0.4 already set, we used the Search Tool for the Retrieval of Interacting Genes (STRING) database version 11.5 (https://string-db.org/) for PPI analysis[21].

Results

Mendelian randomization analysis

Under the previous screening conditions, we found a total of 1624 proteins with cis-pQTL in Europeans and 256 proteins with cis-pQTL in East Asians (Table S5–6, Supplemental Digital Content 1, http://links.lww.com/JS9/D30). After an initial proteome-wide two-sample MR analysis, we identified seven significant protein targets (from the FinnGen consortium) and two significant protein targets (from the BioBank Japan consortium) in two datasets of different ancestry as outcomes. The F-statistics for all the pQTLs ranged from 59.86 to 79366.17; these values are much greater than 10 and represent strong instrumental variable features (Table 1).

For potential targets identified in European populations (Table 1, Fig. 2A, Table S7, Supplemental Digital Content 1, http://links.lww.com/JS9/D30), genetic prediction of elevated plasma FUT3 (OR = 0.87; 95% CI: 0.84–0.89; $P=4.70\times10^{-32}$), UGT 1A6 (OR = 0.68; 95% CI: 0.64–0.73; $P=9.58\times10^{-30}$), NOE1 (OR = 0.58; 95% CI: 0.52–0.66; $P=4.21\times10^{-23}$), and FUT5 (OR = 0.91; 95% CI: 0.89–0.94; $P=6.79\times10^{-12}$) reduced the risk of cholelithiasis. Conversely, increased genetic prediction of GCKR (OR = 2.11; 95% CI: 1,76–2.51; $P=1.38\times10^{-16}$), BGAT (OR = 1.05; 95% CI: 1.03–1.06; $P=4.70\times10^{-15}$), and FKBP52 (OR = 1.75; 95% CI: 1.37–2.24; $P=8.61\times10^{-6}$) increased the risk of cholelithiasis.

For East Asian populations (Table 1, Fig. 2B, Table S8, Supplemental Digital Content 1, http://links.lww.com/JS9/D30), increased plasma KLB levels (OR = 1.11; 95% CI: 1.07–1.16; $P=7.59\times10^{-7}$) increase the risk of cholelithiasis, whereas increased FGFR4 levels (OR = 0.94; 95% CI: 0.91–0.96; $P=4.07\times10^{-6}$) decrease the risk of cholelithiasis. There was also no heterogeneity in the IVW methods based on multiple SNPs (Table S9, Supplemental Digital Content 1, http://links.lww.com/JS9/D30). Additionally, we found that *rs1260326* was significantly associated with triglycerides by retrieving the PhenoScanner database; therefore, we removed GCKR (Table S10, Supplemental Digital Content 1, http://links.lww.com/JS9/D30).

Reverse causality detection

Steiger filtering suggested that all the identified plasma proteins had reliable directionality ($P_{FUT3}=1.49\times10^{-242}$, $P_{UGT\ 1A6}=4.65\times10^{-118}$, $P_{NOE1}=8.37\times10^{-23}$, $P_{BGAT}=4.17\times10^{-259}$, $P_{FUT5}=2.88\times10^{-263}$, $P_{KLB}=3.21\times10^{-13}$, and $P_{FGFR4}=1.31\times10^{-23}$; Table 2, Table S12, Supplemental Digital Content 1, http://links.lww.com/JS9/D30). Reverse MR analysis also did not detect possible reverse confounding factors, revealing robust directionality (Table 2, Table S11, Supplemental Digital Content 1, http://links.lww.com/JS9/D30).

Bayesian colocalization analysis

According to the Bayesian colocalization analysis, we found strong colocalization evidence ($PPH_4>0.8$) for FUT3 ($PPH_4=1.000$), UGT 1A6 ($PPH_4=0.996$), NOE1 ($PPH_4=0.995$), and FKBP52 ($PPH_4=0.972$) in the European population and KLB ($PPH_4=0.970$) and FGFR4 ($PPH_4=0.971$) in the East Asian population (Table 2, Figure S1-6, Supplemental Digital Content 2, http://links.lww.com/JS9/D31). Considering that exposure and outcome are unlikely to share the same genetic locus, BGAT ($PPH_4=1.06\times10^{-6}$) and FUT5 ($PPH_4=2.46\times10^{-17}$) were removed (Table 2, Table S12, Supplemental Digital Content 1, http://links.lww.com/JS9/D30).

Table 1

MR results for European and East Asian significantly associated with cholelithiasis after Bonferroni correction.

Ancestry	Protein	UniProt ID	SNP	Effect allele	Method	OR (95% CI)	*P*	F statistics	Reference
European	FUT3	P21217	rs708686	T	Wald ratio	0.865 (0.845–0.886)	4.70×10^{-32}	1479.658	Ferkingstad *et al.*[7]
European	UGT 1A6	P19224	rs1976391	G	Wald ratio	0.680 (0.496–0.626)	9.58×10^{-30}	759.910	Ferkingstad *et al.*[7]
European	NOE1	Q99784	rs977371848	T	Wald ratio	0.557 (0.496–0.626)	4.21×10^{-23}	174.518	Ferkingstad *et al.*[7]
European	GCKR	Q14397	rs1260326	T	Wald ratio	2.106 (1.765–2.512)	1.38×10^{-16}	106.467	Ferkingstad *et al.*[7]
European	BGAT	P16442	rs950529388	G	Wald ratio	1.046 (1.035–1.058)	4.21×10^{-23}	1479.658	Ferkingstad *et al.*[7]
European	FUT5	Q11128	rs3760775	T	Wald ratio	0.911 (0.887–0.935)	6.79×10^{-12}	1479.658	Ferkingstad *et al.*[7]
European	FKBP52	Q02790	rs34728283	A	Wald ratio	1.749 (1.367–2.238)	8.61×10^{-6}	114.652	Ferkingstad *et al.*[7]
East Asian	KLB	Q86Z14	rs6531719	G	Wald ratio	1.111 (1.066–1.158)	7.59×10^{-7}	59.856	Sun *et al.*[8]
East Asian	FGFR4	P22455	rs351855	A	Wald ratio	0.938 (0.913–0.964)	4.06×10^{-6}	118.233	Sun *et al.*[8]

OR, odds ratio; SNP, single-nucleotide polymorphism (using cis-acting in this research).

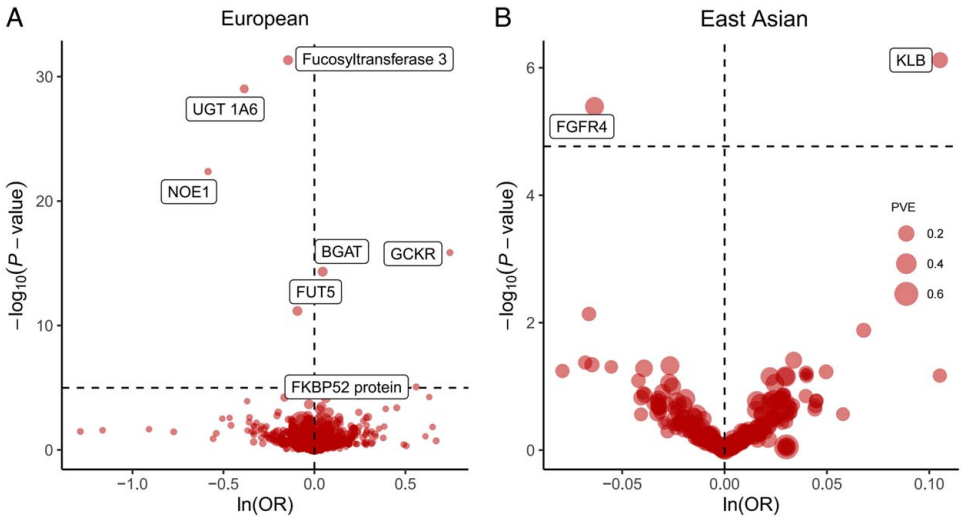

Figure 2. Volcano plots showing drug targets with potentially significant causal effects (based on Bonferroni's method) for (A) Europe and (B) East Asia. OR, increased risk of cholelithiasis for elevated plasma protein levels per SD. PVE, proportion of variance explained.

External validation

We further validated the potential drug targets in European populations using cis-pQTLs from the ARIC and UKB-PPP cohorts (Table S13, Supplemental Digital Content 1, http://links.lww.com/JS9/D30). Both FUT3 and FKBP52 had significant effects on and consistent effects on cholelithiasis. The significant causal effects of genetically predicted FUT3 (OR = 0.87; 95% CI: 0.84–0.91; $P = 1.52 \times 10^{-11}$) and FKBP52 (OR = 1.94; 95% CI: 1.43–2.64; $P = 2.52 \times 10^{-5}$) on cholelithiasis were further confirmed in the UKB-PPP cohort. In addition, FUT3 (OR = 0.998; 95% CI: 0.997–0.999; $P = 1.57 \times 10^{-6}$) also had a significant and directionally consistent effect on the UK biobank (Table S14, Supplemental Digital Content 1, http://links.lww.com/JS9/D30).

Phenome-wide Mendelian randomization

By performing phenome-wide MR analyses in European and East Asian populations, we explored the side effects of the identified drug targets. Considering the safety of potential drug targets, we focused on possible harmful effects. For Europeans, increased circulating plasma NOE1 protein was associated with an increased risk of hemorrhoids (OR = 1.55; 95% CI: 1.39–1.70; $P = 2.25 \times 10^{-8}$), diverticulosis and diverticulitis (OR = 1.46; 95% CI: 1.31–1.61; $P = 5.49 \times 10^{-7}$), and duodenal ulceration (OR = 2.55; 95% CI: 2.13–2.97; $P = 1.56 \times 10^{-5}$) (Table S15, Supplemental Digital Content 1, http://links.lww.com/JS9/D30). Notably, increased FGFR4 levels increased the risk of type 2 diabetes mellitus (OR = 1.05; 95% CI: 1.03–1.06; $P = 7.70 \times 10^{-11}$) in East Asians (Table S16, Supplemental Digital Content 1, http://links.lww.com/JS9/D30).

Protein–protein interaction network

The protein–protein interaction (PPI) network revealed interactions between bile acid metabolism-related proteins cholesterol 7 alpha-hydroxylase (CYP7A1), bile salt export pump (BSEP), Sterol-27-hydroxylase (CYP27A1), and NR1H4 (also known as farnesoid X receptor, FXR) and the targets of four current cholelithiasis drugs of European ancestry (Figure S7, Supplemental Digital Content 2, http://links.lww.com/JS9/D31). Our analysis, based on the comprehensive STRING database, indicated that the interaction between UGT 1A6 and CYP7A1 is the most reliable (known interaction), with interaction evidence supporting these findings. STRING also revealed that UGT 1A6 was coexpressed with NR1H4 and ABCB11, suggesting the relative proximity of these proteins and suggesting their potential relationship with biological processes. However, through the use of the STRING

Table 2

Summary data for sensitivity analyses and external validation results related to possible eight causal proteins.

Ancestry	Protein	UniProt ID	SNP	Bidirectional (IVW-MR)	Steiger filtering	Colocalization PPH_4	Validation
European	FUT3	P21217	rs708686	0.806 (0.586–1.107)	Passed (1.49×10^{-242})	1.000	Statistically significance
European	UGT 1A6	P19224	rs1976391	0.965 (0.863–1.079)	Passed (4.65×10^{-118})	0.996	Statistically significance
European	NOE1	Q99784	rs977371848	1.015 (0.986–1.045)	Passed (8.37×10^{-23})	0.993	Directional consistency
European	GCKR	Q14397	rs1260326	1.029 (0.982–1.078)	Passed (5.10×10^{-14})	0.999	—
European	BGAT	P16442	rs950529388	1.001 (0.978–1.023)	Passed (4.17×10^{-259})	1.06×10^{-6}	—
European	FUT5	Q11128	rs3760775	0.842 (0.675–1.049)	Passed (2.88×10^{-263})	2.46×10^{-17}	—
European	FKBP52	Q02790	rs34728283	0.042 (1.013–0.983)	Passed (2.88×10^{-263})	0.972	Statistically significance
East Asian	KLB	Q86Z14	rs6531719	0.958 (0.621–1.481)	Passed (3.21×10^{-13})	0.970	—
East Asian	FGFR4	P22455	rs351855	0.697 (0.455–1.067)	Passed (1.31×10^{-23})	0.981	—

IVW, inverse variance weighed; MR, Mendelian randomization; PPH_4, posterior probability of hypothesis 4; SNP, single-nucleotide polymorphism.

database, we found no direct clear indications of interactions involving FUT3, NOE1, or FKBP52 (also denoted as FKBP4) with bile acid metabolism-related proteins. Thus, further research is needed to confirm our findings or identify additional interacting partners within the metabolic machinery.

For two potential cholelithiasis drug targets of Asian ancestry, string-based PPI analyses revealed proven interactions between KLB and FGFR4 and between KLB and NR1H4 (Figure S8, Supplemental Digital Content 2, http://links.lww.com/JS9/D31). Additionally, analysis through the STRING database predicted the potential associations of these genes with CYP7A1, BSEP, and CYP27A1, revealing a complex interaction landscape with potential therapeutic implications.

Discussion

In the current study, MR and colocalization analyses revealed the potentially causal effect of the six target genes on cholelithiasis. Specifically, FKBP52, FUT3, NOE1, and UGT 1A6 showed evidence of effects in individuals of European ancestry, while FGFR4 and KLB showed evidence of an effect in individuals of East Asian ancestry.

High expression of FKBP52 (encoded by the FKBP4 gene) may promote the progression of cholelithiasis (Fig. 3). FKBP52 enhances glucocorticoid receptor (GR) activity, which in turn modulates FXR (encoded by the NR1H4 gene) transcription, establishing a mechanism likely provoking cholestasis[22,23]. FXR is pivotal for bile acid homeostasis and influences its synthesis, conjugation, and transport. At the molecular level, FKBP52 has been shown to potentiate the GR-mediated transcriptional repression of small heterodimer partner (SHP), a nuclear receptor that is integral to FXR signaling for bile acid homeostasis. Hence, the upregulation of FKBP52 bolstered GR signaling, thus modulating FXR and incapacitating its role in bile acid regulation. This interference affects essential FXR targets that maintain bile acid equilibrium, such as BSEP (encoded by the ABCB11 gene) and CYP7A1[22–26]. This implies that FKBP52 affects bile acid metabolism through some indirect mechanism.

Our MR study revealed that FUT3 would be a therapeutic target that significantly reduces the risk of cholelithiasis. FUT3 is a member of the glycosyltransferase family. Although the precise role of FUT3 in gallstone development has not been determined, its known involvement in inflammation (leukocyte recruitment and immune responses) would affect bile composition and gallbladder function[27,28]. Additionally, FUT3 could impact gallstone formation by influencing gallbladder epithelial cell adhesion, which may affect cholesterol crystal interactions and nucleation[27]. FUT3's coexpression with FUT2 correlates with increased hepatocyte nuclear Factor 4 alpha (HNF4A) levels, which are crucial for expressing the CYP7A1 gene, which is integral for converting cholesterol to bile acids[29–32]. HNF4A-induced CYP7A1 expression can counteract cholesterol supersaturation, thereby reducing the likelihood of gallstone formation[29,30,33]. This gene interaction could suggest a biological feedback mechanism whereby the expression of FUT3, potentially linked with HNF4A, influences bile acid synthesis, contributing to cholesterol homeostasis and reducing the incidence of cholelithiasis.

NOE1, which is primarily present in the nervous system and is critical for embryonic neural crest cell development, may influence endocrine amine precursor uptake and decarboxylation (APUD) cells—those derived from neural crest cells[34,35]. NOE1 may impact the APUD system, which involves endocrine cells processing amine precursors into hormones, affecting organs such as the hypothalamus. Dysfunction of APUD cells may lead to obesity and disturb gallbladder functions, contributing to an increased risk of cholelithiasis[36,37]. However, multiple validation sets yielded inconsistent results, necessitating caution in asserting the potential for NOE1 as a therapeutic target. Therefore, further research is justified to fully elucidate the role of NOE1 in cholelithiasis.

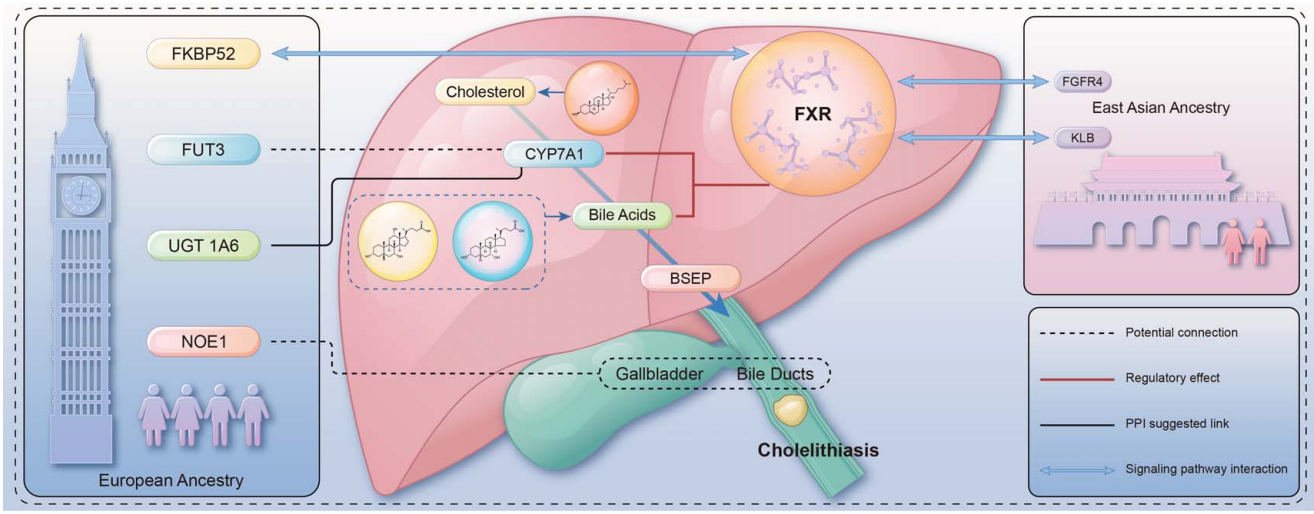

Figure 3. Bile acid metabolism may be a potential pathway for treating cholelithiasis. Dysregulated bile acid metabolism can contribute to the pathogenesis of cholelithiasis by promoting the formation of gallstones. In hepatic cells, bile acid synthesis is regulated by CYP7A1, whose expression is suppressed by FXR-mediated induction of SHP. FXR also modulates the expression of BSEP, which is critical for bile acid secretion. Among the drug targets identified, FUT3 is potentially connected with CYP7A1. PPI analysis suggested that UGT 1A6 is associated with both CYP7A1 and BSEP. The potential link of NOE1 to biliary ducts warrants further investigation. The involvement of FKBP52, FGFR4, KLB, and FXR in signal transduction pathways highlights their relevance as therapeutic targets.

Notably, we found that the increase in UGT 1A6 expression appears to confer protection from cholelithiasis. These findings align with previous studies citing the significance of glucuronidation pathways in the metabolism of bilirubin and various xenobiotics, factors intimately related to bile composition and gallstone formation[38]. In the context of UGT 1A1, its ability to promote the conjugation of bile acids with glucuronic acid can result in more hydrophilic compounds that are less likely to contribute to stone formation and can be more readily excreted[39–42]. Drawing from similar hepatic detoxification pathways as UGT 1A1, UGT 1A6 may exert protective effects against cholelithiasis by facilitating the glucuronidation and subsequent excretion of bile constituents[43]. Notably, our PPI analysis also revealed that UGT 1A6 was associated with key proteins involved in bile acid synthesis and metabolism, namely, CYP7A1, CYP27A1, and BSEP, which are integral components of the intricate bile acid regulatory regime, and that their expression levels influence critical facets of gallstone disease pathophysiology. Taken together, UGT 1A6 may play a protective role in the pathogenesis of cholelithiasis through these pathways.

Our MR analysis also highlighted FGFR4 as a protective factor against cholelithiasis in Asian populations. FGFR4 is a member of the fibroblast growth factor receptor (FGFR) family and is expressed in multiple tissues and is involved in various diseases[44]. It regulates cell function by binding with fibroblast growth factors (FGFs) which can be activated by FXR signaling pathway. Activation of FXR leads to an increase in the expression of FGF15/19, which are actually different species versions of the same protein, with FGF15 being expressed in mice, while FGF19 being expressed in human. FGF15/19 binds to FGFR4 and can modify the synthesis of cholesterol and bile acids while promoting the excretion of bile acids into the gallbladder[45,46]. Therefore, activation of the FXR-FGF15/19-FGFR4 cascade is important for regulating bile acid balance and preventing the formation of cholesterol gallstones[47].

KLB is implicated in obesity-related mechanisms in humans and other mammals. It predominantly functions as a high-affinity coreceptor for FGFs, with a particular affinity for FGF19 and FGF21[48]. KLB is intricately involved in the downstream effects of FXR activation through FGF19-mediated signaling pathways[48]. Additionally, the role of KLB in conjunction with the metabolic actions of FGF21 has a complex influence on obesity, a recognized risk factor for gallstone formation[49]. The effects of FGF21 on adipose tissue metabolism contribute to weight regulation[50]. Therefore, KLB may indirectly predispose individuals to cholelithiasis through obesity-associated changes in BA composition and gallbladder function[51].

Finally, we further conducted PheW-MR to explore potential side effects when protein-targeted therapies were applied for cholelithiasis, which revealed a connection between the NOE1 protein and gastrointestinal bleeding. NOE1 may interact with cells in the APUD system and be involved in regulating angiogenesis and platelet function, thus affecting the health and stability of gastrointestinal blood vessels. Moreover, our PheW-MR analysis suggested a link between FGFR4 and type 2 diabetes, which may be achieved by regulating insulin sensitivity and adipose tissue function. Considering the potential side effects of NOE1 and FGFR4, additional caution may be needed when these proteins are used as drug targets.

In addition, PheW-MR showed that reducing FKBP52 levels in the European population and KLB levels in the Asian population and increasing FUT3 and UGT 1A6 levels in the European population may be potential therapeutic targets for treating cholelithiasis without significant side effects.

Of note, this MR analysis revealed differences in therapeutic targets for cholelithiasis between East Asian and European populations. The bulk of GWAS data have been collected from individuals of European descent, while people of other ancestries are underrepresented. As a result, polymorphic traits associated with diseases and responses to drugs—what we term QTLs—are more comprehensively identified and characterized in European cohorts. In contrast, the quantity of such data for East Asian populations is considerably more limited. This paucity of data inevitably leads to an incomplete picture of the genetic architecture that underlies cholelithiasis in East Asians, manifesting in a disparity of identified targets compared with those found in European populations. In addition, disparities also stem from genetic differences, varying diets, and differences in gut microbiome diversity. Polymorphic variations in target genes such as UGT 1A6, FUT3, and FGFR4 differ in prevalence between ethnicities, affecting disease susceptibility and drug response[52–54]. Dietary differences with Western diets high in refined carbohydrates and cholesterol and traditional Asian diets rich in fiber and low in saturated fats, may influence bile lithogenicity. Additionally, the gut microbiome is crucial for bile acid circulation, with variations in microbial composition between populations possibly leading to distinct bile acid profiles and impacting gallstone development and treatment options[55].

Limitations

Despite the insights gained from our comparative analysis for potential therapeutic targets for cholelithiasis in European and East Asian populations, we must acknowledge certain limitations within our study. First, upon rigorous screening for suitable pQTLs within both European and East Asian populations using strict criteria, we were unable to identify overlap in the pQTLs associated with the significant drug targets. The heterogeneous nature of the available data contributes to discrepancies in the identified potential therapeutic targets. The preponderance of genomic data derived from individuals of European descent contrasts with the underrepresentation of East Asian cohorts, leading to a potential skew in the landscape of pharmacological interventions, highlighting the need for more inclusive genomic databases. Second, our analysis focused predominantly on plasma protein targets without considering nonprotein molecules and did not examine hepatic tissue, which may provide key insights into the pathogenesis of gallstones. Future studies should seek to encompass a broader range of biological samples, including hepatic tissue, which plays a central role in bile production and metabolism, thus potentially uncovering novel targets for therapeutic intervention. Finally, although our study identified several candidate protein targets, the intricate mechanisms underlying their roles in cholelithiasis remain to be elucidated. It is essential to recognize that MR cannot fully recapitulate a clinical trial. MR mimics lifelong, low-dose exposure to a drug and assumes a linear relationship between exposure and outcome. This differs from a clinical trial, where typically higher doses of a drug are studied over a shorter period.

Consequently, the results from MR might not directly equate to the actual effect size seen in practice, and these identified drug targets need to be validated in preclinical and clinical settings to confirm their efficacy and safety. Therefore, while our findings offer a valuable starting point, they necessitate substantiation through rigorous basic scientific research to validate the feasibility of the potential therapeutic strategies for gallstone disease, particularly within diverse ethnic groups.

Conclusions

Our MR study revealed that genetically predicted levels of the plasma proteins FUT3, NOE1, UGT1A6, and FKBP52 have potential as prospective targets in Europeans, whereas the plasma levels of KLB and FGFR4 may serve as potential targets for the treatment of cholelithiasis in East Asians. Further basic and clinical studies are justified to determine the underlying mechanisms involved and verify the feasibility of developing novel therapy based on these findings.

Ethical approval

Not applicable.

Consent

Not applicable.

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Author contribution

Z.A.N. and Z.S.T.: conceived the hypothesis; L.X.D and S.L.B.: designed the study, analyzed the data, and drafted the manuscript; S.L.B.: interpreted the results; L.X.D.: was responsible for data management; Z.A.N. and Z.S.T.: reviewed and revised the manuscript. All authors approved the final version of the manuscript.

Conflicts of interest disclosure

None of the authors has any conflict of interest to disclose.

Research registration unique identifying number (UIN)

1. Name of the registry: not applicable.
2. Unique identifying number or registration ID: not applicable.
3. Hyperlink to your specific registration (must be publicly accessible and will be checked): not applicable.

Guarantor

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Data availability statement

The data that support the findings of this study are available in the Additional files of this article.

Provenance and peer review

Not applicable.

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