*nutrients*

MDPI

*Article*

# The Causal Relationship Between Choline Metabolites and Acute Acalculous Cholecystitis: Identifying *ABCG8* as Colocalized Gene

**Yuntong Gao [1,†], Kun Mao [1,†], Congying Yang [1], Xisu Wang [1], Shixuan Liu [1], Zimeng Ma [1], Qi Zhai [1], Liang Shi [1], Qian Wu [1,*] and Tianxiao Zhang [1,2,*]**

[1] Department of Epidemiology and Biostatistics, School of Public Health, Xi'an Jiaotong University Health Science Center, Xi'an 710061, China; altogik2002@stu.xjtu.edu.cn (Y.G.); submacar@stu.xjtu.edu.cn (K.M.); yangcy022@stu.xjtu.edu.cn (C.Y.); doofzijing@outlook.com (X.W.); liushixuan2022@gmail.com (S.L.); mzmbla0321@163.com (Z.M.); zhaiqi0722@163.com (Q.Z.); shi-liang@stu.xjtu.edu.cn (L.S.)

[2] National Anti-Drug Laboratory, Shaanxi Regional Center, Xi'an 712000, China

* Correspondence: wuqian@xjtu.edu.cn (Q.W.); joshuaz@mail.xjtu.edu.cn (T.Z.); Tel.: +86-029-8265-5108 (T.Z.)

† These authors contributed equally to this work.

**Abstract:** Background: Acute acalculous cholecystitis (AAC) is a type of cholecystitis with high mortality rate while its pathogenesis remains complex. Choline is one of the essential nutrients and is related to several diseases. This study aimed to explore the causal relationship between choline metabolites and AAC and its potential mechanisms. Methods: This research utilized the two-sample Mendelian randomization method to investigate the causal relationship between choline metabolites and AAC. Additionally, multivariable Mendelian randomization and mediated Mendelian randomization were used to explore potential confounding effects from low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TGs), and coronary artery disease (CAD). Linkage disequilibrium score regression (LDSC), co-localization analysis, and enrichment analysis were used to investigate relevant molecular mechanisms. Results: There is a negative causal relationship between total choline (OR [95%CI] = 0.9982 [0.9974, 0.9990], $p = 0.0023$), phosphatidylcholine (OR [95%CI] = 0.9983 [0.9976–0.9991], $p = 0.0040$), sphingomyelin (OR [95%CI] = 0.9980 [0.9971–0.9988], $p = 0.0001$), and AAC. The mediating effects of LDL were −0.0006 for total choline, −0.0006 for phosphatidylcholine, and −0.0008 for sphingomyelin, indicating a protective effect of total choline, phosphatidylcholine, and sphingomyelin on AAC. Colocalized SNP rs75331444, which is mapped to gene *ABCG8*, was identified for total choline (PPH4 = 0.8778) and sphingomyelin (PPH4 = 0.9344). Conclusions: There is a causal relationship between choline metabolites and cholecystitis, mediated through the protective action of LDL. Our results suggest that *ABCG8* may play a role in the development of non-calculous cholecystitis.

**Keywords:** Mendelian randomization; choline metabolites; acute acalculous cholecystitis; low-density lipoprotein; *ABCG8*

check for updates

**Citation:** Gao, Y.; Mao, K.; Yang, C.; Wang, X.; Liu, S.; Ma, Z.; Zhai, Q.; Shi, L.; Wu, Q.; Zhang, T. The Causal Relationship Between Choline Metabolites and Acute Acalculous Cholecystitis: Identifying *ABCG8* as Colocalized Gene. *Nutrients* **2024**, *16*, 3588. https://doi.org/10.3390/nu16213588

Academic Editor: Nicholas M. Morton

Received: 16 September 2024
Revised: 8 October 2024
Accepted: 9 October 2024
Published: 22 October 2024

**Copyright:** © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

## 1. Introduction

Cholecystitis is one of the most common digestive diseases occurring as a complication with cholelithiasis, affecting 10% of the global population [1]. Acute cholecystitis may occur with gallstones or without gallstones [2]. The latter case is called acute acalculous cholecystitis (AAC), accounting for 2–15% of all cases of acute cholecystitis [3]. The pathogenesis of AAC is complex. Ischemia–reperfusion injury, along with the involvement of proinflammatory eicosanoid mediators, is considered the core of this process [4]. Cholestasis, opioid therapy, positive-pressure ventilation, and total parenteral nutrition have also been considered contributing factors [5]. AAC is characterized by a significant mortality rate and is related to various complications [6]. Therefore, further research is needed on the pathogenic mechanisms of AAC.

Choline is an essential human nutrient involved in a variety of biological functions, including neurotransmission, membrane synthesis, lipid transport, and one-carbon metabolism [7]. It plays an important role in human growth and development and has been negatively associated with a variety of diseases, including birth defects, neurodevelopmental and cognitive alterations, hepatic steatosis, cardiovascular disease (CVD), and cancer [7]. Choline exists in several forms, including water-soluble free choline, fat-soluble phosphatidylcholine, and sphingomyelin [8]. A protective causal relationship between sphingomyelin and cholelithiasis was suggested in previous studies [9], while the relationship between choline and its metabolites and AAC has not been established.

Mendelian randomization is a research approach that uses randomly assigned genetic variants as instrumental variables to infer causal relationships between exposures and outcomes [10]. In addition, co-localization is an essential analytical method for exploring the common causal molecular mechanism between different phenotypes [11]. In this study, we aimed to explore the underlying causal relationship between choline and its metabolites and cholecystitis in vivo through Mendelian randomization analyses, and to further test the genetic correlation and identify colocalized genes.

## 2. Materials and Methods

### *2.1. Study Design*

Mendelian randomization (MR) is a method used in epidemiology and genetics to investigate the causal relationship between an exposure (such as a risk factor or treatment) and an outcome (such as a disease). The key idea of MR is to identify genetic variants (typically single nucleotide polymorphisms [SNPs]) that serve as instrumental variables (IVs), and these variants need to meet three conditions: (1) the variant is associated with the exposure; (2) the variant is not associated with the outcome via a confounding pathway; and (3) the variant does not directly affect the outcome, only possibly indirectly via the exposure [12]. By examining the association between the selected DNA variants and the outcome, the potential causal relationship between the exposures and the outcome is determined [13]. In the present study, we implemented two-sample Mendelian randomization (TSMR) of which the relationships of IVs with exposures and outcomes were measured from two samples [14].

### *2.2. Data Sources*

We took three choline metabolites as our exposure factors from UK Biobank (n = 114,999), including total choline, phosphatidylcholine, and sphingomyelin, and we downloaded the data through the IEU Open GWAS database (https://gwas.mrcieu.ac.uk) (10 November 2023) [15]. SNP-Cholecystitis (ICD-10 code K81) data were taken from the Neale lab in the UK Biobank (UKB) (http://www.nealelab.is/uk-biobank) (10 November 2023). In the multivariable MR analysis, we extracted data for four additional variables: low-density lipoprotein (LDL): ieu-b-5089 (n = 201,678); high-density lipoprotein (HDL): ieu-b-109 (n = 403,943) [16]; triglycerides (TGs): ieu-b-111 (n = 441,016) [16]; and coronary vascular disease (CAD): ebi-a-GCST90013868 (n = 352,063) [17]. Multiple inter-institutional selection was attempted to avoid sample overlap between exposures and outcomes, and all samples used in this study were obtained from the European population. These published data have undergone quality control and ethical review and are ready for immediate use.

### *2.3. Two-Sample Mendelian Randomization Analysis*

We selected SNPs with strong correlations with exposures and outcomes at the $p < 5 \times 10^{-8}$ level and further removed variants in linkage disequilibrium ($r^2 < 0.001$ over a scan window of 10,000 kb). In addition, F-tests were performed and SNPs with F-static values <10 were excluded. Finally, we identified 49 SNPs for total choline, 48 SNPs for phosphatidylcholine, and 48 SNPs for sphingomyelin, which were used in subsequent Mendelian randomization analyses.

Five methods including MR Egger [18], weighted median [19], inverse-variance-weighted (IVW) [20], simple mode, and weighted mode [21] were utilized to model the causal relationship between exposures and outcomes. A significant causal relationship is considered when the *p*-value of IVW is <0.05 and the results of the five methods have the same direction. The results were tested for heterogeneity using the Cochran Q test [22]. The MR-Egger intercept [23] and the MR presso global test [24] were used to test for pleiotropy. If heterogeneity and/or pleiotropy were detected, the MR presso outlier method [25] was implemented to remove outliers. Sensitive analysis was performed based on the IVW results through leave-one-out tests for each SNP [25]. In addition, to control the potential confounding effects, multivariable Mendelian randomization analyses were performed. LDL, HDL, TGs, and CAD were included as covariates. The IVW random-effects model and MR-Egger model were utilized for TSMR analysis.

### *2.4. Reverse and Mediated Mendelian Randomization Analysis*

To further elucidate the causal role of LDL, we conducted mediated Mendelian randomization analysis with LDL as the mediating factor. Initially, we examined the reverse Mendelian randomization results; total choline, phosphatidylcholine, and sphingomyelin were selected as the outcomes; and cholecystitis was selected as the exposure factor. In the mediator of Mendelian randomization analysis, we first employed a two-sample Mendelian randomization approach to estimate the effect of total choline, phosphatidylcholine, and sphingomyelin on LDL ($\beta_1$). Subsequently, using LDL as the exposure and cholecystitis as the outcome, we estimated the direct effect of the mediator on cholelithiasis ($\beta_2$). The indirect effect of sphingomyelin on cholelithiasis through the mediator was calculated using $\beta_1 \times \beta_2$, and its significance was tested using a stepwise testing method.

### *2.5. Linkage Disequilibrium Score Regression (LDSC) and Co-Localization Analysis*

The LDSC analysis was performed to investigate the genetic correlation between three choline metabolites and AAC [26]. Co-localization analysis was implemented using the coloc package to further examine the potential shared causal variants between the three choline metabolites and AAC [13].

### *2.6. Gene Enrichment Analysis*

The gene list was extracted based on SNPs identified from co-localization analysis. The STRING database (https://string-db.org) (22 November 2023) was utilized to obtain a set of genes which are based on protein–protein interaction (PPI) data [27]. Gene set enrichment analysis was then performed for this gene set using GO and KEGG databases [28].

## 3. Results

### *3.1. Causal Relationship between Choline Metabolites and AAC*

The analysis pipeline of the present study is shown in Figure 1. A significant causal relationship was identified between the three choline metabolites and AAC. The IVW results indicated a significant negative causal relationship between total choline (OR [95%CI] = 0.9982 [0.9974, 0.9990], $p$ = 0.0023), phosphatidylcholine (OR [95%CI] = 0.9983 [0.9976–0.9991], $p$ = 0.0040), sphingomyelin (OR [95%CI] = 0.9980 [0.9971–0.9988], $p$ = 0.0001), and AAC (Figure 2). Neither significant heterogeneity (Cochran Q test, $p > 0.05$) nor pleiotropy (MR-Egger global test, $p > 0.05$) was detected (Supplementary Table S1). The results of leave-one-out tests and funnel plots are summarized in Supplementary Figures S2 and S3. No individual SNPs were identified to dictate the IVW results. The funnel plots were approximately symmetrical. To further validate the causal relationship identified from TSMR, multivariable Mendelian randomization analyses were further performed. The serum levels of HDL, LDL, TGs, and CAD were adjusted and the results are summarized in Table 1. Interestingly, the causal relationship between all the three choline metabolites and AAC did not remain significant after being adjusted for the serum level of LDL (total choline: OR [95%CI] = 0.9985 [0.9978,0.9991], $p$ = 0.1153); phosphatidylcholine (OR [95%CI]

= 0.9989 [0.9984,0.9994], $p$ = 0.2219); or sphingomyelin (OR [95%CI] = 0.9983 [0.9976,0.9991], $p$ = 0.1660). Significant horizontal pleiotropy was identified using the MR-Egger intercept in the multivariable models adjusting for HDL and TGs. The overall F value for the selected instrumental variables was greater than 10 in all groups (Supplementary Table S2).

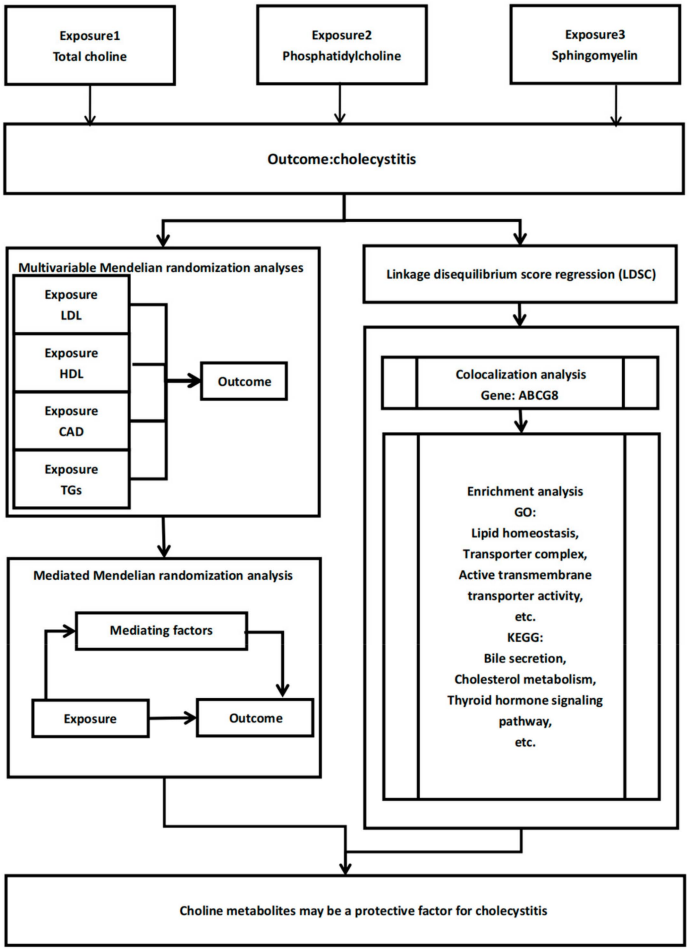


**Figure 1.** Conceptual framework of this study.

| Exposure | Methods | OR (95% CI) | p |
|---|---|---|---|
| Total choline | MR Egger | 0.9971 (0.9959 to 0.9984) | 0.0160 |
| | Weighted median | 0.9979 (0.9970 to 0.9988) | 0.0130 |
| | Inverse variance weighted | 0.9982 (0.9974 to 0.9990) | 0.0023 |
| | Simple mode | 0.9998 (0.9998 to 0.9999) | 0.9158 |
| | Weighted mode | 0.9981 (0.9973 to 0.9989) | 0.0426 |
| Phosphatidylcholine | MR Egger | 0.9974 (0.9963 to 0.9985) | 0.0150 |
| | Weighted median | 0.9982 (0.9974 to 0.9990) | 0.0252 |
| | Inverse variance weighted | 0.9983 (0.9976 to 0.9991) | 0.0040 |
| | Simple mode | 0.9993 (0.9990 to 0.9996) | 0.6169 |
| | Weighted mode | 0.9983 (0.9976 to 0.9990) | 0.0471 |
| Sphingomyelin | MR Egger | 0.9980 (0.9971 to 0.9989) | 0.0126 |
| | Weighted median | 0.9980 (0.9972 to 0.9989) | 0.0082 |
| | Inverse variance weighted | 0.9980 (0.9971 to 0.9988) | 0.0001 |
| | Simple mode | 0.9983 (0.9976 to 0.9991) | 0.1883 |
| | Weighted mode | 0.9980 (0.9971 to 0.9989) | 0.0070 |




**Figure 2.** Analysis of the relationship between choline metabolites and cholecystitis by Mendelian randomization analysis (results corresponding to five different methods).

**Table 1.** Multivariable Mendelian randomization analysis between choline metabolites and cholecystitis.

| Exposure | nSNPs | MVMR-IVW | | MVMR-Egger | | *p* for MR-Egger Intercept | F Statistic |
|---|---|---|---|---|---|---|---|
| | | OR (95%CI) | *p* | OR (95%CI) | *p* | | |
| HDL | 321 | 0.9994 (0.9992 to 0.9997) | 0.3446 | 1.0014 (1.0008 to 1.0020) | 0.0916 | 0.0003 | 17.3554 |
| Total choline | | 0.9977 (0.9967 to 0.9987) | 0.0051 | 0.9975 (0.9965 to 0.9986) | 0.0022 | | 11.1154 |
| LDL | 90 | 0.9984 (0.9976 to 0.9991) | 0.0618 | 0.9983 (0.9975 to 0.9990) | 0.0895 | 0.867 | 52.8175 |
| Total choline | | 0.9985 (0.9978 to 0.9991) | 0.1153 | 0.9984 (0.9977 to 0.9991) | 0.1243 | | 47.1951 |
| Triglyceride | 278 | 1.0005 (1.0003 to 1.0008) | 0.3097 | 0.9994 (0.9991 to 0.9997) | 0.4261 | 0.0393 | 114.9307 |
| Total choline | | 0.9984 (0.9978 to 0.9991) | 0.0192 | 0.9984 (0.9977 to 0.9991) | 0.0137 | | 22.0579 |
| CAD | 88 | 0.9996 (0.9994 to 0.9998) | 0.263 | 0.9997 (0.9995 to 0.9998) | 0.5529 | 0.8148 | 29.6231 |
| Total choline | | 0.9979 (0.9969 to 0.9988) | 0.0001 | 0.9979 (0.9970 to 0.9988) | 0.0001 | | 82.2872 |
| HDL | 322 | 0.9992 (0.9989 to 0.9996) | 0.2120 | 1.0013 (1.0007 to 1.0018) | 0.1218 | 0.0002 | 19.9792 |
| Phosphatidylcholine | | 0.9981 (0.9973 to 0.9989) | 0.0140 | 0.9979 (0.9970 to 0.9988) | 0.0006 | | 12.8171 |
| LDL | 91 | 0.9981 (0.9973 to 0.9989) | 0.0299 | 0.9979 (0.9970 to 0.9988) | 0.0440 | 0.7143 | 68.9316 |
| Phosphatidylcholine | | 0.9989 (0.9984 to 0.9994) | 0.2219 | 0.9988 (0.9983 to 0.9993) | 0.2040 | | 58.3947 |
| Triglyceride | 280 | 1.0006 (1.0003 to 1.0008) | 0.2737 | 0.9995 (0.9993 to 0.9997) | 0.5045 | 0.0480 | 107.0640 |
| Phosphatidylcholine | | 0.9986 (0.9979 to 0.9992) | 0.0229 | 0.9985 (0.9979 to 0.9992) | 0.0185 | | 24.7822 |
| CAD | 86 | 0.9996 (0.9994 to 0.9997) | 0.2560 | 0.9997 (0.9996 to 0.9999) | 0.6550 | 0.6491 | 29.8555 |
| Phosphatidylcholine | | 0.9982 (0.9974 to 0.9990) | 0.0003 | 0.9982 (0.9975 to 0.9990) | 0.0007 | | 91.7388 |
| HDL | 316 | 0.9993 (0.9990 to 0.9996) | 0.2730 | 1.0014 (1.0008 to 1.0021) | 0.0929 | 0.0002 | 29.3628 |
| Sphingomyelin | | 0.9978 (0.9968 to 0.9987) | 0.0029 | 0.9978 (0.9968 to 0.9988) | 0.0026 | | 16.1736 |
| LDL | 90 | 0.9989 (0.9985 to 0.9994) | 0.3740 | 0.9988 (0.9983 to 0.9993) | 0.3520 | 0.7679 | 23.9513 |
| Sphingomyelin | | 0.9983 (0.9976 to 0.9991) | 0.1660 | 0.9983 (0.9975 to 0.9990) | 0.1590 | | 24.7277 |
| Triglyceride | 277 | 1.0000 (1.0000 to 1.0000) | 0.9573 | 0.9989 (0.9984 to 0.9994) | 0.1858 | 0.0829 | 77.8575 |
| Sphingomyelin | | 0.9977 (0.9967 to 0.9987) | 0.0005 | 0.9975 (0.9965 to 0.9986) | 0.0002 | | 26.9886 |
| CAD | 91 | 0.9997 (0.9996 to 0.9998) | 0.528 | 1.0006 (1.0003 to 1.0009) | 0.3730 | 0.0679 | 28.0342 |
| Sphingomyelin | | 0.9976 (0.9965 to 0.9986) | 0.0001 | 0.9977 (0.9967 to 0.9987) | 0.0002 | | 83.5995 |

MVMR-Egger: multivariable Mendelian randomization using Egger regression; MVMR-IVW: multivariable Mendelian randomization using inverse-variance-weighted approach; nSNPs: number of SNPs used in MR; LDL: low-density lipoprotein; HDL: high-density lipoprotein; CAD: coronary artery disease. The grayish-white background shows where the adjust variable is applied and makes the table intuitive.

### *3.2. Reverse and Mediator Mendelian Randomization Analysis*

The IVW results of the reverse Mendelian randomization analysis revealed no significant causal relationship between cholecystitis and total choline ($p = 0.2520$), phosphatidylcholine ($p = 0.3424$), or sphingomyelin ($p = 0.1100$). When LDL was considered as the outcome, significant positive causal relationships were observed with total choline ($\beta_1 = 0.3533$, $p = 1.66 \times 10^{-16}$), phosphatidylcholine ($\beta_1 = 0.3468$, $p = 2.17 \times 10^{-28}$), and sphingomyelin ($\beta_1 = 0.4682$, $p = 1.31 \times 10^{-51}$). Additionally, a significant inverse causal relationship was found when LDL was the exposure and cholecystitis was the outcome ($\beta_2 = -0.0018$, $p = 0.0022$). The mediating effects of LDL were $-0.0006$ for total choline, $-0.0006$ for phosphatidylcholine, and $-0.0008$ for sphingomyelin. A causal steps approach confirmed the significance of LDL's mediating effect in the causal relationships between

total choline ($p$ = 0.0041), phosphatidylcholine ($p$ = 0.0031), and sphingomyelin ($p$ = 0.0027) with cholecystitis.

### *3.3. Genetic Correlation between the Three Choline Metabolites and AAC*

Although no genome-wide genetic correlations were detected between the three choline metabolites and AAC (Table 2), a significant colocalized SNP, rs75331444, was identified for total cholines (PPH4 = 0.8778) and sphingomyelin (PPH4 = 0.9344) (Figure 3). This SNP is mapped to gene *ABCG8*. No colocalization signal was detected in the colocalization analysis of phosphatidylcholine and AAC (Supplementary Tables S3 and S4).

**Table 2.** Genetic correlation between choline metabolites and cholecystitis.

| Exposure | Outcome | Rg | *p* |
|---|---|---|---|
| Total choline | Cholecystitis | −0.0940 | 0.6372 |
| Phosphatidylcholine | Cholecystitis | −0.1077 | 0.5836 |
| Sphingomyelin | Cholecystitis | −0.2796 | 0.1645 |

Rg: genetic correlation.

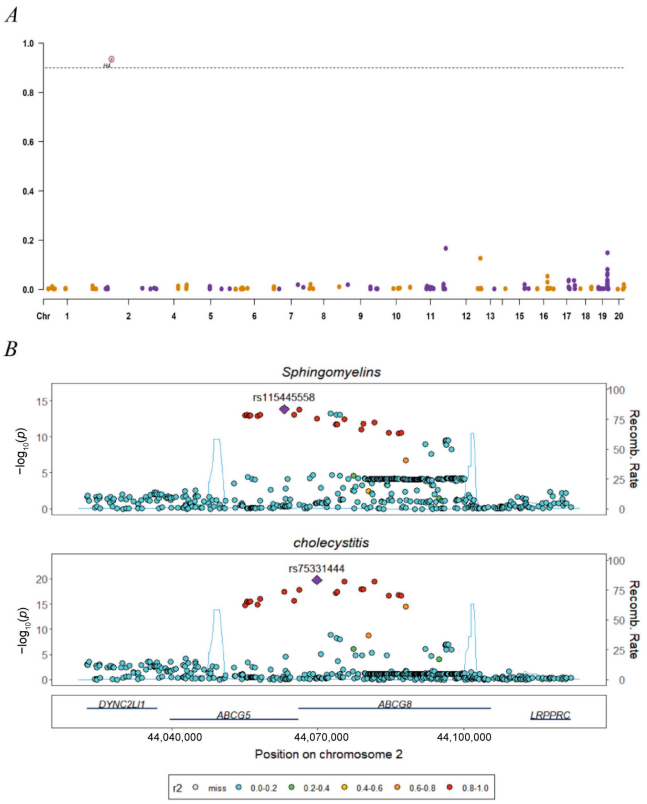


**Figure 3.** Manhattan plots for colocalization analysis. (**A**) Manhattan plot of selected SNP associations with PPH4 at the genome-wide scale with yellow and purple dots to distinguish between adjacent chromosomal locations. (**B**) Locus comparison plot for the COLOC analysis of the notable colocalization regions (PPH4 > 0.9).

### *3.4. Enrichment Pathway for ABCG8*

The colocalized gene *ABCG8* was found to be enriched in multiple biological processes, cellular components, and molecular functions in the GO database (Figure 4A). In the KEGG database, this gene was identified to be enriched in bile secretion, cholesterol metabolism, the thyroid hormone signaling pathway, ABC transporters, and proximal tubule bicarbonate reclamation (Figure 4B).

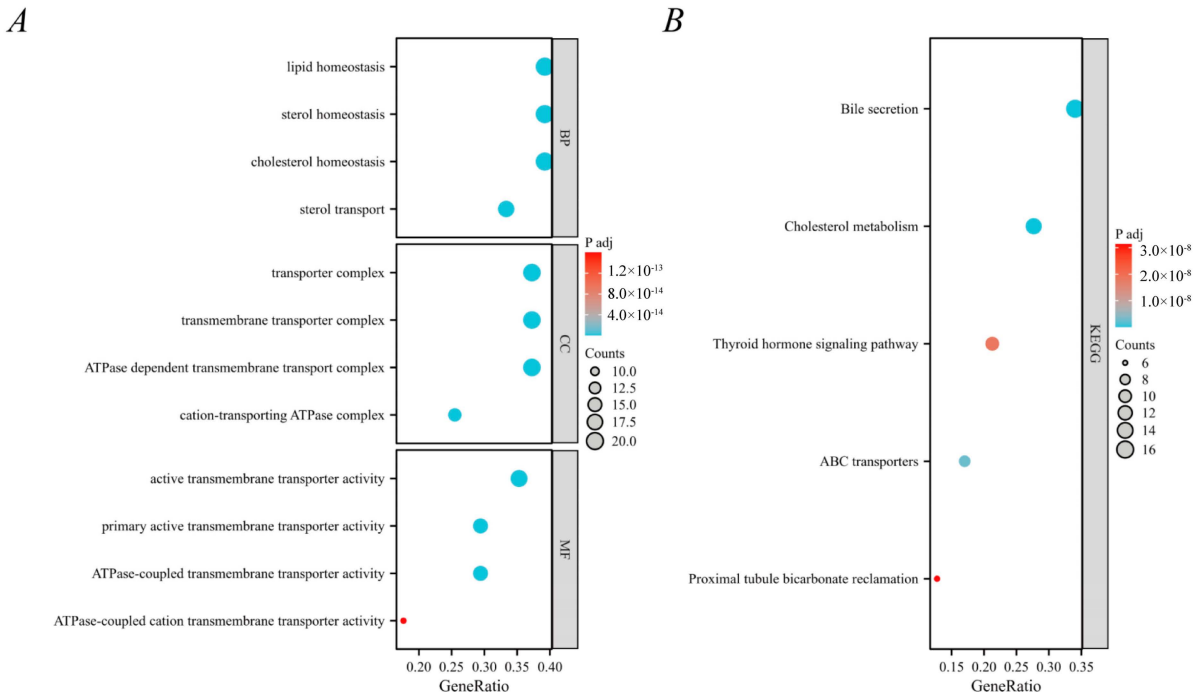


**Figure 4.** Bubble plots for enrichment analysis of genes and their related genes obtained from colocalization analysis. (**A**) GO enrichment analysis, including biological processes (BPs), cellular components (CCs), and molecular functions (MFs). (**B**) KEGG enrichment analysis.

## 4. Discussion

Choline, a trace component of plasma, is involved in various key physiological functions in the body and drives disease progression. The causal relationship between plasma levels of total choline, phosphatidylcholine, and sphingomyelin and cholecystitis was evaluated utilizing Mendelian randomization, LDSC, co-localization analysis, and enrichment analysis. The two-sample Mendelian randomization results indicated that total choline, phosphatidylcholine, and sphingomyelin had a protective effect on AAC.

Multivariable Mendelian randomization was applied to assess the reliability of two-sample Mendelian randomization analysis and explore the sources of causality. In the multivariable Mendelian randomization results, it was observed that all positive results became negative after adjusting LDL. This suggested that LDL is prone to be an important confounder in this process, which was not seen with other possible confounders (HDL, triglycerides, and CAD). The results of mediated Mendelian randomization suggest that high levels of LDL are a protective factor for AAC, and choline may enhance this effect. This also explains the non-significant results in multivariable analysis after adjusting for LDL levels. Previous study has found a minor protective effect of serum low-density lipoprotein (LDL) cholesterol on cholecystitis, which is partly in accordance with this study. [29] LDL has been shown to interact with the immune system in several studies. [30] It has been shown that LDL can reduce lipopolysaccharide mediated central and peripheral inflammation [31] and bacterial infection may be one of the etiologies of AAC, but its specific role in cholecystitis still lacks specialized research support. Alternatively, LDL is involved in bile acid metabolism [32], which is responsible for the transport of cho-lesterol outward into the liver tissue, and cholestasis is one of the etiologies of AAC.

Moreover, choline retains its independent protective effect, potentially linked to the expression levels of certain proteins. To explore the source of the causal relationship between choline and cholecystitis, LDSC and colocalization were used. In LDSC analysis, there were co-localization sites between total choline, sphingomyelin, and cholecystitis. The results of the colocalization analysis suggested that there may be a particular driver molecule between total choline, sphingomyelin, and cholecystitis. Sphingomyelin accounts

for a high proportion of total choline in the body [33]. In our results, both co-localization analyses had the same site, and the co-localization intensity of total choline was lower than that of sphingolipids. This suggested that the positive co-localization result of total choline with cholecystitis may be due to sphingolipids.

*ABCG8* was localized through colocalization analysis. It may play an important role in the causal relationship between choline metabolites and cholecystitis. *ABCG8* is exclusively expressed in hepatocytes, gallbladder epithelial cells, and intestinal cells, where it interacts with the ATP-binding cassette transporter G5 (ABCG5), which forms a specialized heterodimer involved in sterol metabolism [34]. Common mutations in *ABCG8* confer most of the genetic risk for cholelithiasis, accounting for approximately 25% of the total risk [35]. Previous studies have shown that *ABCG8* expression is elevated in patients with cholesterol gallstone disease and cholecystitis [36], and our study further demonstrated that *ABCG8* also plays a role in the development of non-calculous cholecystitis. This suggests that the molecular mechanisms between cholelithiasis and non-calculous cholecystitis have some similarities. Previous studies have demonstrated the role of ABCG8 in excretion of cholesterol [37,38]. Taken together, choline metabolites may play a protective role against AAC by promoting cholesterol excretion and transport. The specific molecular mechanism still needs further animal experiments. Cholestasis is one of the possible pathogenic factors of AAC. This strengthens the causal relationship between choline and AAC. Current animal studies have focused on choline with liver [39,40]. There are few animal studies on the relationship between choline and ACC. Animal experiments are needed in the future.

This research also has some limitations. Our study focused on the European population, making it difficult to extrapolate the findings to other populations. We did not analyze all choline metabolites, due to missing data, which may bring a new perspective to this study. The current research reveals causal reference, and wouldn't have great significance for clinical practice. Related clinical translational research is needed in the future.

## 5. Conclusions

In summary, our study, for the first time, confirms a causal relationship between choline metabolites and cholecystitis, mediated through the protective action of LDL, and identifies the possible loci responsible for this causal relationship, suggesting a role for *ABCG8* in the development of non-calculous cholecystitis, which provides valuable information for probing its molecular mechanisms. Moreover, this finding could provide significant insights for individuals regarding their nutritional intake, especially the parenterally nourished patients.

**Supplementary Materials:** The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/nu16213588/s1. Supplementary Figure S1: Scatter plots of the association between choline metabolites and cholecystitis. (A) Total choline and cholecystitis, (B) phosphatidylcholine and cholecystitis, and (C) sphingolipids and cholecystitis. Supplementary Figure S2: MR leave-one-out sensitivity analysis for choline metabolites on cholelithiasis. (A) Total choline and cholecystitis, (B) phosphatidylcholine and cholecystitis, and (C) sphingolipids and cholecystitis. Supplementary Figure S3: Funnel plots of Mendelian randomization (MR) analyses between choline metabolites and cholelithiasis. (A) Total choline and cholecystitis, (B) phosphatidylcholine and cholecystitis, and (C) sphingolipids and cholecystitis. Supplementary Table S1: Results of Mendelian randomization analysis in five methods and tests of heterogeneity (Cochran Q test) and tests of multiple validity (MR-Egger intercept and global test). Supplementary Table S2: Results of multivariable Mendelian randomization in IVW method and MR-Egger method and tests of multiple validity (MR-Egger intercept) and F-statistic. Supplementary Table S3: Results of colocalization analysis between sphingomyelin and cholecystitis. Supplementary Table S4: PPH4 values for each SNP within significant regions in the results of colocalization analysis between sphingomyelin and cholecystitis. Supplementary Table S5: Results of mediated Mendelian randomization analysis in five methods and tests of heterogeneity (Cochran Q test) and tests of multiple validity (MR-Egger intercept and global test).

**Author Contributions:** Conceptualization, methodology, validation, and formal analysis, Y.G. and K.M.; data curation, X.W., Z.M. and Q.Z.; writing—original draft preparation, Y.G., K.M. and C.Y.; writing—review and editing, T.Z. and S.L.; visualization, Y.G. and L.S.; supervision, T.Z., Q.W. and S.L.; project administration, T.Z. and Q.W. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was supported by the Hygiene and Health Care Scientific Research Program of Shaanxi Province (2022D010) and Scientific Research Program of Shaanxi provincial center for disease control and prevention (HXDSH20232686). The funding body did not participate in the design, conduct, or writing of the study.

**Institutional Review Board Statement:** UK Biobank data have approval from the North West Multicenter Research Ethics Committee (MREC) (REC reference: 21/NW/0157) (18 June 2021). The data we used do not contain individual patient information, so no additional ethical review was required.

**Informed Consent Statement:** We used data from publicly available databases that have been ethically reviewed without individual information, so no additional informed consent was required.

**Data Availability Statement:** The original data presented in this study are openly available in UK Biobank (https://www.ukbiobank.ac.uk/) (10 November 2023)—low-density lipoprotein (LDL): ieu-b-5089 (n = 201,678); high-density lipoprotein (HDL): 6ieu-b-109 (n = 403,943) [16]; triglycerides (TGs): ieu-b-111 (n = 441,016) [16]; and coronary vascular disease (CAD): ebi-a-GCST90013868 (n = 352,063) [17].

**Acknowledgments:** The authors would like to give thanks to members of Neale lab for their analysis.

**Conflicts of Interest:** All authors declare no conflicts of interests. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

## References

1. Jia, F.; Ma, Y.; Liu, Y. Association of milk consumption with the incidence of cholelithiasis disease in the US adult population. *BMC Public Health* **2023**, *23*, 1639. [CrossRef] [PubMed]
2. O'Connor, O.J.; Maher, M.M. Imaging of Cholecystitis. *Am. J. Roentgenol.* **2011**, *196*, W367–W374. [CrossRef] [PubMed]
3. Elwood, D.R. Cholecystitis. *Surg. Clin. N. Am.* **2008**, *88*, 1241–1252. [CrossRef]
4. Frazee, R.C.; Nagorney, D.M.; Mucha, P. Acute acalculous cholecystitis. *Mayo Clin. Proc.* **1989**, *64*, 163–167. [CrossRef]
5. Barie, P.S.; Eachempati, S.R. Acute acalculous cholecystitis. *Curr. Gastroenterol. Rep.* **2003**, *5*, 302–309. [CrossRef]
6. Indar, A.A.; Beckingham, I.J. Acute cholecystitis. *Br. Med. J.* **2002**, *325*, 639–643. [CrossRef]
7. Wiedeman, A.M.; Barr, S.I.; Green, T.J.; Xu, Z.; Innis, S.M.; Kitts, D.D. Dietary Choline Intake: Current State of Knowledge across the Life Cycle. *Nutrients* **2018**, *10*, 1513. [CrossRef]
8. Leermakers, E.T.; Moreira, E.M.; Jong, J.C.K.-D.; Darweesh, S.K.; Visser, T.; Voortman, T.; Bautista, P.K.; Chowdhury, R.; Gorman, D.; Bramer, W.M.; et al. Effects of choline on health across the life course: A systematic review. *Nutr. Rev.* **2015**, *73*, 500–522. [CrossRef]
9. Mi, J.; Jiang, L.; Liu, Z.; Wu, X.; Zhao, N.; Wang, Y.; Bai, X. Identification of blood metabolites linked to the risk of cholelithiasis: A comprehensive Mendelian randomization study. *Hepatol. Int.* **2022**, *16*, 1484–1493. [CrossRef]
10. Davies, N.M.; Holmes, M.V.; Smith, G.D. Reading Mendelian randomisation studies: A guide, glossary, and checklist for clinicians. *BMJ-Br. Med. J.* **2018**, *362*, k601. [CrossRef]
11. Wu, Y.; Broadaway, K.A.; Raulerson, C.K.; Scott, L.J.; Pan, C.; Ko, A.; He, A.; Tilford, C.; Fuchsberger, C.; E Locke, A.; et al. Colocalization of GWAS and eQTL signals at loci with multiple signals identifies additional candidate genes for body fat distribution. *Hum. Mol. Genet.* **2019**, *28*, 4162–4173. [CrossRef] [PubMed]
12. Emdin, C.A.; Khera, A.V.; Kathiresan, S. Mendelian Randomization. *JAMA-J. Am. Med. Assoc.* **2017**, *318*, 1925–1926. [CrossRef] [PubMed]
13. Giambartolomei, C.; Vukcevic, D.; Schadt, E.E.; Franke, L.; Hingorani, A.D.; Wallace, C.; Plagnol, V. Bayesian Test for Colocalisation between Pairs of Genetic Association Studies Using Summary Statistics. *PLoS Genet.* **2014**, *10*, e1004383. [CrossRef] [PubMed]
14. Guo, J.; Peng, C.; He, Q.; Li, Y. Type 2 diabetes and the risk of synovitis-tenosynovitis: A two-sample Mendelian randomization study. *Front. Public Health* **2023**, *11*, 1142416. [CrossRef] [PubMed]
15. Lyon, M.S.; Andrews, S.J.; Elsworth, B.; Gaunt, T.R.; Hemani, G.; Marcora, E. The variant call format provides efficient and robust storage of GWAS summary statistics. *Genome Biol.* **2021**, *22*, 32. [CrossRef]
16. Richardson, T.G.; Sanderson, E.; Palmer, T.M.; Ala-Korpela, M.; Ference, B.A.; Smith, G.D.; Holmes, M.V. Evaluating the relationship between circulating lipoprotein lipids and apolipoproteins with risk of coronary heart disease: A multivariable Mendelian randomisation analysis. *PLoS Med.* **2020**, *17*, e1003062. [CrossRef]

17. Mbatchou, J.; Barnard, L.; Backman, J.; Marcketta, A.; Kosmicki, J.A.; Ziyatdinov, A.; Benner, C.; O'dushlaine, C.; Barber, M.; Boutkov, B.; et al. Computationally efficient whole-genome regression for quantitative and binary traits. *Nat. Genet.* **2021**, *53*, 1097–1103. [CrossRef]
18. Bowden, J.; Smith, G.D.; Burgess, S. Mendelian randomization with invalid instruments: Effect estimation and bias detection through Egger regression. *Int. J. Epidemiol.* **2015**, *44*, 512–525. [CrossRef]
19. Bowden, J.; Smith, G.D.; Haycock, P.C.; Burgess, S. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. *Genet. Epidemiol.* **2016**, *40*, 304–314. [CrossRef]
20. Lawlor, D.A.; Harbord, R.M.; Sterne, J.A.C.; Timpson, N.; Smith, G.D. Mendelian randomization: Using genes as instruments for making causal inferences in epidemiology. *Stat. Med.* **2008**, *27*, 1133–1163. [CrossRef]
21. Hartwig, F.P.; Smith, G.D.; Bowden, J. Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. *Int. J. Epidemiol.* **2017**, *46*, 1985–1998. [CrossRef] [PubMed]
22. Araujo, H.A.; Cooper, A.B.; Hassan, M.A.; Venditti, J. Estimating suspended sediment concentrations in areas with limited hydrological data using a mixed-effects model. *Hydrol. Process.* **2012**, *26*, 3678–3688. [CrossRef]
23. Burgess, S.; Thompson, S.G. Interpreting findings from Mendelian randomization using the MR-Egger method. *Eur. J. Epidemiol.* **2017**, *32*, 377–389. [CrossRef] [PubMed]
24. Verbanck, M.; Chen, C.-Y.; Neale, B.; Do, R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat. Genet.* **2018**, *50*, 693–698. [CrossRef]
25. Hemani, G.; Tilling, K.; Smith, G.D. Orienting the causal relationship between imprecisely measured traits using GWAS summary data. *PLoS Genet.* **2017**, *13*, e1007081.
26. Ni, G.; Moser, G.; Wray, N.R.; Lee, S.H.; Ripke, S.; Neale, B.M.; Corvin, A.; Walters, J.T.; Farh, K.-H.; Holmans, P.A.; et al. Estimation of Genetic Correlation via Linkage Disequilibrium Score Regression and Genomic Restricted Maximum Likelihood. *Am. J. Hum. Genet.* **2018**, *102*, 1185–1194. [CrossRef]
27. Szklarczyk, D.; Gable, A.L.; Nastou, K.C.; Lyon, D.; Kirsch, R.; Pyysalo, S.; Doncheva, N.T.; Legeay, M.; Fang, T.; Bork, P.; et al. The STRING database in 2021: Customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res.* **2021**, *49*, D605–D612. [CrossRef]
28. Kanehisa, M.; Goto, S. KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res.* **2000**, *28*, 27–30. [CrossRef]
29. Yang, H.; Chen, L.; Liu, K.; Li, C.; Li, H.; Xiong, K.; Li, Z.; Lu, C.; Chen, W.; Liu, Y. Mendelian randomization rules out the causal relationship between serum lipids and cholecystitis. *BMC Med Genomics* **2021**, *14*, 224. [CrossRef]
30. Rhoads, J.P.; Major, A.S. How Oxidized Low-Density Lipoprotein Activates Inflammatory Responses. *Crit Rev Immunol.* **2018**, *38*, 333–342. [CrossRef]
31. Radford-Smith, D.E.; Yates, A.G.; Rizvi, L.; Anthony, D.C.; Probert, F. HDL and LDL have distinct, opposing effects on LPS-induced brain inflammation. *Lipids Heal. Dis.* **2023**, *22*, 54. [CrossRef] [PubMed]
32. Sato, R. Recent advances in regulating cholesterol and bile acid metabolism. *Biosci. Biotechnol. Biochem.* **2020**, *84*, 2185–2192. [CrossRef] [PubMed]
33. Hammad, S.M.; Lopes-Virella, M.F. Circulating Sphingolipids in Insulin Resistance, Diabetes and Associated Complications. *Int. J. Mol. Sci.* **2023**, *24*, 14015. [CrossRef] [PubMed]
34. Patel, S.B.; Graf, G.A.; Temel, R.E. ABCG5 and ABCG8: More than a defense against xenosterols. *J. Lipid Res.* **2018**, *59*, 1103–1113. [CrossRef] [PubMed]
35. Lammert, F.; Gurusamy, K.; Ko, C.W.; Miquel, J.F.; Méndez-Sánchez, N.; Portincasa, P.; Van Erpecum, K.J.; Van Laarhoven, C.J.; Wang, D.Q. Gallstones. *Nat. Rev. Dis. Primers* **2016**, *2*, 16024. [CrossRef] [PubMed]
36. Yoon, J.H.; Choi, H.S.; Jun, D.W.; Yoo, K.-S.; Lee, J.; Yang, S.Y.; Kuver, R. ATP-Binding Cassette Sterol Transporters Are Differentially Expressed in Normal and Diseased Human Gallbladder. *Dig. Dis. Sci.* **2013**, *58*, 431–439. [CrossRef]
37. Coy, D.J.; Wooton-Kee, C.R.; Yan, B.; Sabeva, N.; Su, K.; Graf, G.; Vore, M. ABCG5/ABCG8-independent biliary cholesterol excretion in lactating rats. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2010**, *299*(1), G228–G235. [CrossRef]
38. Yu, X.-H.; Qian, K.; Jiang, N.; Zheng, X.L.; Cayabyab, F.S.; Tang, C.K. ABCG5/ABCG8 in cholesterol excretion and atherosclerosis. *Clin. Chim. Acta.* **2014**, *428*, 82–88.
39. Miyachi Y, Akiyama K, Tsukuda Y, Kumrungsee T, Yanaka N Liver choline metabolism and gene expression in choline-deficient mice offspring differ with gender. *Biosci. Biotechnol. Biochem.* **2021**, *85*, 447–451. [CrossRef]
40. Mehedint, M.G.; Zeisel, S.H. Choline's role in maintaining liver function: New evidence for epigenetic mechanisms. *Curr. Opin. Clin. Nutr. Metab. Care* **2013**, *16*, 339–345. [CrossRef]


**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.