Causal associations between changes in lipid profiles and risk of gallstone disease: a two-sample Mendelian randomization study

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Background: Nonalcoholic fatty liver disease (NAFLD) has been linked to gallstone disease (GSD) in observational studies; however, the relationships between certain lipid profiles and GSD remain unclear.

Methods: We adopted a two-sample Mendelian randomization (MR) framework by applying different statistical methods to assess causalities between lipid profiles and GSD. We identified single-nucleotide polymorphisms (SNPs) for blood lipids and NAFLD from separate previous genome-wide association studies (GWASs).

Results: We retrieved GSD SNPs attributed to 10,520 cases and 361,194 controls and validated our estimates using GWAS summary data from UK Biobank. We also performed sex-stratified analyses. Based on the summary estimates of 41, 59, 35, and 2 SNPs for low-density lipoprotein cholesterol (LDLC), high-density lipoprotein cholesterol (HDLC), triglycerides (TGs), and NAFLD, respectively, we found no evidence of a causal relationship between genetically-predicted lipid profiles and GSD. The odds ratios were 0.995 for LDLC [95% confidence interval (CI): 0.994–0.998] per 0.98 mmol/L, 0.999 for HDLC (95% CI: 0.996–1.003) per 0.41 mmol/L, 0.997 for TGs (95% CI: 0.994–1.001) per 1 mmol/L, and 0.993 for NAFLD (95% CI: 0.984–1.003). No evidence of associations between lipid profile s and GSD in validation MR analyses or the sex-stratification analyses was noted.

Conclusions: Genetically predicted hyperlipidemia or NAFLD is not causally associated with GSD.

Keywords: Blood lipids; nonalcoholic fatty liver disease (NAFLD); gallstone disease (GSD); Mendelian randomization (MR)

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Introduction

Gallstone disease (GSD), also known as cholelithiasis, is one of the most common and costly known gastrointestinal diseases (1-3) and affects 10.5–15% of the population in the developed world (4). The prevalence of GSD varies by race, with the highest (48%) seen among Native Americans and Hispanics, the lowest (5%) recorded in African populations, and midrange figures reported in Asian populations (5–20%) (5-10). GSD is the most common digestive disease leading to hospital admissions in Europe and the USA. An estimated 1.8 million ambulatory care visits result in diagnosis of GSD annually, with an associated treatment cost of $6.2 billion in the USA (11). Complications of GSD include cholecystitis, cholangitis, and pancreatitis. In addition, GSD is an important risk factor for gallbladder cancer (12) and is associated with significant complications and poor patient prognosis. Consequently, reducing the prevalence of GSD may also yield benefits in the clinical treatment of gallbladder cancer.

It is known that obesity is a risk factor for GSD (1,2), and obesity tends to be associated with unhealthily high levels of blood lipids and fatty liver disease (3,4). The association between hyperlipidemia and GSD is, however, controversial. Several clinical studies, mostly observational investigations and systematic reviews, have reported a positive correlation between hyperlipidemia and GSD (13,14). However, an epidemiological study found that blood lipid profiles did not differ in patients with and without GSD (5). Meanwhile, Ferkingstad *et al.* reported that blood lipid levels of low-density lipoprotein cholesterol (LDLC) are not causative factors in gallstone formation (15). Most obese individuals suffer from nonalcoholic fatty liver disease (NAFLD), and previous clinical retrospective observational studies have reported that NAFLD is an independent risk factor for GSD (16,17). Importantly, observational research can easily be influenced by confounding factors and sample size, and stronger evidence is needed to verify the relationship between lipid profiles in the blood or liver and GSD. Drugs to reduce lipid levels are widely used for asymptomatic GSD patients, but the use of these drugs is linked to many side effects, and further research is needed to guide treatment (18).

Mendelian randomization (MR) is a useful method by which causal associations may be inferred through the adoption of genetic information such as single-nucleotide polymorphisms (SNPs) or copy number variations as instrumental variables to test for causality (19-21). MR takes advantage of the random segregation of alleles inherited by offspring from their parents during meiosis. An MR study is analogous to random allocation of the treatment in a randomized controlled trial and can overcome both reverse causation and confounding (22). In this study, we sought to identify any causal relationships between lipid profiles in the blood or liver and GSD using the MR method in 2 steps. First, we used two-sample Mendelian randomization (TSMR) analysis to estimate the causal effect of lipid profiles on GSD. Second, we validated the estimates using one-sample MR analysis. We present the following article in accordance with the STREGA reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-21-4007/rc).

Methods

Genome-wide association studies (GWASs) data of blood lipids

We selected genetic variants that were associated with blood lipids, including LDLC, high-density lipoprotein cholesterol (HDLC), and triglycerides (TGs), at a genome-wide significance level in the Global Lipids Genetics Consortium (GLGC) (23) covering data from 60 studies. We selected summary estimates of 126 SNPs that (I) have been shown to be associated with blood lipids in the GLGC GWAS ($P<5\times10^{-8}$) and included 188,577 participants (90% European ancestry), and that (II) were independent variants, using data from the 1000 Genomes Project (linkage disequilibrium threshold of $r^2<0.001$ and located 1 Mb apart from each other (Tables S1-S3). A detailed description of the statistical methods and quality-control efforts was provided in a previous publication by the GLGC (23). The effect sizes were calculated with respect to the effect allele per 1 standard deviation increase in the plasma lipid level (which was equal to 0.98 mmol/L for LDLC, 0.41 mmol/L for HDLC, and 1 mmol/L for TGs).

GWAS data of NAFLD

NAFLD ranges from hepatic steatosis to steatohepatitis and, finally, to fibrosis. Computed tomography can be used to measure hepatic steatosis, while steatohepatitis or fibrosis must be assessed histologically. We selected the significant SNPs ($P<5\times10^{-8}$) associated with hepatic steatosis and histologic NAFLD from the largest-to-date GWAS study (24,25). Patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) rs738409 and

transmembrane 6 superfamily member 2 (*TM6SF2*) rs58542926, the 2 strongest genetic predictors of NAFLD, were used as proxies for hepatic steatosis and histologic NAFLD (25). Because rs58542926 was not genotyped in most of the GWAS summary data used in this investigation, rs2228603 at the *NCAN* gene locus, which exists in strong linkage disequilibrium with rs58542926 (pairwise R^2=0.76 based on the phase III data of the 1000 Genomes Project in European individuals) and which is significantly associated with liver fat content (26), was used in place of *TM6SF2* rs58542926.

GWAS data of LDLC, HDLC, TGs, and GSD in UK Biobank

We used data from UK Biobank, one of the largest available prospective cohort study databases, which includes more than 500,000 participants (aged 40–69 years) recruited between 2006 and 2010. The biochemical assays, genotyping, and follow-up of the study design have been published elsewhere (27). UK Biobank GWAS results are available for 371,714 unrelated individuals of European ancestry from Neale Lab (http://www.nealelab.is/uk-biobank/). Genetic associations of both sexes in combination and individually, together with LDLC, HDLC, and TGs, were obtained for validation analyses, where the associations (sex, age, age-squared, the interaction of sex and age, and the interaction of sex and age-squared) were discerned via multivariable linear regression adjusted for the first 20 principal components (28). The trait phenotypes for LDLC, HDLC, and TGs can be found on the UK Biobank showcase using codes 30780, 30706, and 30870, respectively. Unfortunately, the sample size for the NAFLD phenotype present in UK Biobank was insufficient for us to have any confidence in the results, so we did not make use of the NAFLD phenotype data from UK Biobank.

Genetic associations of both sexes in combination and individually with GSD were obtained from UK Biobank summary statistics provided by Neale Lab (Cambridge, MA, USA) as outcomes. The GSD phenotype could be found as part of the International Classification of Diseases, 10th revision code listings on the UK Biobank showcase using code 41202.

Statistical analysis

The instrument variables were first assessed to discern whether they were robustly associated with their lipid traits by computing the proportion of variance explained and the *F* score values. For MR estimation with LDLC, HDLC, TGs, and NAFLD as the exposure variables and GSD as the outcome variable, MR-pleiotropy residual sum and outlier (MR-PRESSO) was used to identify and remove outliers at a P value <0.05. After dropping the outliers, we harmonized the summary data from the exposure and outcome parameters to ensure that the effect of an SNP on the exposure and the effect of the same SNP on the outcome each corresponded to the same allele (29). We employed 4 different methods to estimate the causal association between the lipid profiles and GSD: inverse variance-weighting (IVW) (random-effects model), MR-Egger, weighted median, and simple median. We adopted Cochran's Q test to assess the heterogeneity. In addition to the heterogeneity test, we used the MR-Egger regression method to test for horizontal pleiotropy (30). Heterogeneity can be revealed by a scatterplot, while horizontal pleiotropy can be represented by a forest plot and funnel plot. We considered the association as causal if the directions of the estimates were consistently determined by at least 3 methods. Furthermore, we performed one-sample MR analyses using the LDLC, HDLC, TGs, and GSD GWAS summary data of combined genders from UK Biobank as a validation data set. To conduct sex-stratified analyses, we performed one-sample MR analysis on female- or male-specific GWAS summary data of LDLC, HDLC, TGs, and GSD from UK Biobank with the same SNPs chosen as instrument variables as were used in the previous TSMR analysis.

In addition to the 4 different MR methods, leave-one-out sensitivity analysis was conducted to test the robustness of the MR estimation by excluding a single variant from the analysis at a time. The fluctuation of the estimates in response to this exclusion reflected the influence of the variant in the causal estimation.

Notably, some of the instrument variables used in the previous MR analyses were associated with more than one lipid profile. Meanwhile, multivariable MR has an advantage over univariate MR in that it accounts for potential pleiotropic influence. We conducted multivariable MR using the IVW method to estimate the direct causal effect of LDLC, HDLC, and TGs on the outcomes by applying the method to the complete set of 126 lipid-associated SNPs. All MR analyses were performed using the "MendelianRandomization", "TwoSampleMR", and "MRPRESSO" packages in R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria).

Table 1 Characteristics of Global Lipids Genetics Consortium and UK Biobank datasets

Exposure/outcome	Datasets	No. SNPs	Sample size (No. of cases)	Population
LDL-cholesterol	GLGC	41	83,198	90% European
HDL-cholesterol	GLGC	59	92,860	90% European
Triglycerides	GLGC	35	91,598	90% European
Hepatic steatosis	GOLD	2	7,176	100% European
Histological NAFLD	AGES	2	2,868	100% European
LDL-cholesterol	UK Biobank	41	343,621	100% European
HDL-cholesterol	UK Biobank	59	315,133	100% European
Triglycerides	UK Biobank	35	343,992	100% European
Main outcome				
Gallstone disease	UK Biobank		371,714 (10,520)	100% European

LDL, low-density lipoprotein; HDL, high-density lipoprotein; NAFLD, non-alcoholic fatty liver disease; GLGC, Global Lipids Genetics Consortium; GOLD, Genetics of Obesity-related Liver Disease; AGES, Age, Gene/Environment Susceptibility-Reykjavik Study; SNPs, single-nucleotide polymorphisms.

Ethics statement

The GWAS summary data used for MR analyses in this investigation are publicly available (23,24). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Results

No causal effect of hyperlipidemia on GSD

The characteristics of the populations included in the GLGC and UK Biobank are shown in *Table 1*. We first selected SNPs that could serve as valid instrumental variables for each blood lipid (LDL, HDL, and TGs) in the European population based on association summary statistics from the GLGC study. From the GLGC study, following MR-PRESSO and harmonization correction, we obtained a total of 41, 59, and 35 index SNPs to serve as instrumental variables for LDL, HDL, and TGs, respectively (Tables S1,S2,S4). The selected SNPs in total explained 6.90%, 3.67%, or 4.27% of the observed phenotypic variance for LDL, HDL, or TGs, respectively. Importantly, the *F* score values for these SNPs were 150.3, 59.9, and 116.7, respectively, all of which were larger than 10, suggesting that the selected SNPs had a sufficiently strong effect to serve as valid instruments and that weak instrument bias was unlikely to occur.

In UK Biobank, we identified 10,520 participants with GSD and subsequently obtained association summary statistics of GSD from UK Biobank for the selected instrumental variables of the blood lipids. To investigate the potential association between blood lipids and GSD, we applied 4 different methods to complete TSMR analyses (*Table 2*, Figures S1-S4). The IVW analysis indicated a marginal negative association between the LDLC level and GSD [odds ratio (OR) 0.995; 95% confidence interval (CI): 0.994–0.998; P<0.001]. Meanwhile, no evidence was found for a causal relationship between the HDLC level and GSD (OR 0.999, 95% CI: 0.996–1.003; P=0.731) or the TGs level and GSD (OR 0.997, 95% CI: 0.994–1.001; P=0.146). These results suggested that genetically predicted blood lipid levels were not associated with GSD. The results of the TSMR analyses were consistent in the four methods.

Cochran's Q test indicated that there was significant heterogeneity for LDLC and HDLC (*Table 2*). However, the leave-one-out analyses did not materially change the results of the TSMR estimate. The funnel and forest plots showed an absence of directional pleiotropy, with a symmetrical distribution of variant effects (Figures S4-S12). To validate the estimate, we performed one-sample MR analyses with the identified SNPs using the GWAS summary data of LDLC, HDLC, TGs, and GSD from UK Biobank. The resultant findings were similar to those of the TSMR analyses (*Figure 1*, Table S5).

Female sex has been identified as a risk factor for GSD (31). To investigate whether any of the 3 blood lipids showed

Table 2 Two-sample Mendelian randomization estimations showing the effect of lipids on GSD

Exposure	Methods	Odds ratio[a]	95% CI		P value	Ph	Q-statistics
			Lower limit	Upper limit			
LDLC	IVW	0.996	0.993	0.998	5.46E–04	9.06E–03	64.1
	MR-Egger	0.995	0.992	0.999	7.97E–03	7.33E–03	63.8
	Weighted median	0.997	0.994	1.000	4.25E–02	–	–
	Simple median	0.997	0.993	1.001	1.06E–01	–	–
	MR-Egger intercept[b]	0.0001	–0.0002	0.0003	6.57E–01	–	–
HDLC	IVW	0.999	0.996	1.003	7.31E–01	2.76E–04	102.6
	MR-Egger	0.997	0.989	1.004	3.51E–01	2.82E–04	101.2
	Weighted median	0.997	0.993	1.002	2.48E–01	–	–
	Simple median	0.998	0.993	1.003	4.06E–01	–	–
	MR-Egger intercept[b]	0.0002	–0.0002	0.0005	3.77E–01	–	–
Triglycerides	IVW	0.997	0.994	1.001	1.46E–01	3.79E–01	35.9
	MR-Egger	0.993	0.987	0.999	2.98E–02	4.70E–01	32.9
	Weighted median	0.998	0.993	1.003	4.17E–01	–	–
	Simple median	1.003	0.996	1.009	4.39E–01	–	–
	MR-Egger intercept[b]	0.0001	0.0000	0.0005	9.45E–02	–	–
Hepatic steatosis	IVW	0.994	0.985	1.003	2.06E–01	4.63E–03	8.0
Histologic NAFLD	IVW	0.993	0.984	1.003	1.53E–01	8.88E–03	6.8

[a], odds ratio per 1 SD increase; [b], regression coefficient (95% CI). GSD, gallstone disease; CI, confidence interval; LDLC, low-density lipoprotein cholesterol; HDLC, high-density lipoprotein cholesterol; NAFLD, non-alcoholic fatty liver disease; Ph, P value for heterogeneity; SD, standard deviation; IVW, inverse variance-weighting; MR, Mendelian randomization.

evidence of sex-specific effects, we performed a sex-stratified MR analysis on sex-specific GWAS data from UK Biobank. No evidence was found to support an association between blood lipids and GSD in either men or women (*Figure 1*, *Tables 3*,*4*). No heterogeneity or pleiotropy was apparent between blood lipids and GSD in either sex (Figures S1-S3). In summary, our MR study did not support a causal association between hyperlipidemia and GSD.

In the leave-one-out analysis, we confirmed that no single genetic variant was strongly driving the overall effect of each lipid profile on GSD (Figures S13-S15). In the multivariable MR analysis that adjusted for the effect of each blood lipid, the results remained unchanged (*Figure 2*, Table S6). The multivariable-adjusted β values were 0.002 (95% CI: –0.001 to 0.005; P=0.261) for LDLC, 0.000 (95% CI: –0.006 to 0.006; P=0.983) for HDLC, and 0.005 (95% CI: –0.002 to 0.013; P=0.148) for TGs (Table S2).

No causal effect of NAFLD on GSD

We used 2 well-established hepatic steatosis-associated variants as genetic instruments to test the causal effect of hepatic steatosis on GSD (Table S4). The 2 SNPs explained 3.2% of the variance in hepatic steatosis and the mean F sore value was 118.56. With only 2 SNPs used as instrument variables, we performed a conventional MR analysis using the IVW method on GSD (Figures S4,S8). As listed in *Table 2*, we observed no significant association between genetically instrumented hepatic steatosis and GSD (OR 0.994, 95% CI: 0.985–1.003; P=0.206).

We further tested whether a genetically increased risk for histologic NAFLD has a different effect on GSD as compared to that of hepatic steatosis. Consistent with the results of hepatic steatosis, however, no significant causal relationship was found between genetically driven histologic NAFLD and GSD (*Table 2*). Taken together, the results of

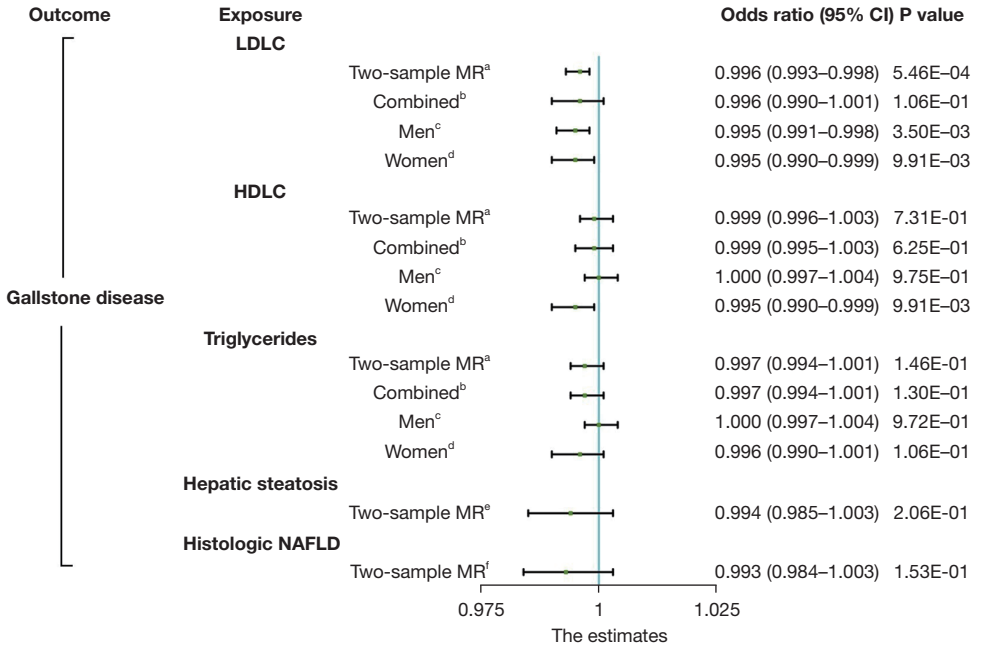

Figure 1 Comparison of the total causal estimations with heterogeneity and pleiotropic effect between lipid profiles and gallstone disease risk being considered via Mendelian randomization. [a], two-sample MR analysis of the Global Lipids Genetics Consortium study and the UK Biobank cohort; [b], one-sample MR analysis of all participants in the UK Biobank cohort; [c], one-sample MR analysis of male participants in the UK Biobank cohort; [d], one-sample MR analysis of female participants in the UK Biobank cohort; [e], two-sample MR analysis of the Genetics of Obesity-Related Liver Disease study and the UK Biobank cohort; [f], two-sample MR analysis of the Age, Gene/Environment Susceptibility-Reykjavik study and the UK Biobank cohort. CI, confidence interval; LDLC, low-density lipoprotein cholesterol; HDLC, high-density lipoprotein cholesterol; NAFLD, non-alcoholic fatty liver disease; MR, Mendelian randomization.

our MR study did not support a causal association between NAFLD and GSD.

Discussion

To our knowledge this was the first large-scale study to assess the causal relationship between lipid profiles in the blood or liver and GSD, and our results suggest that hyperlipidemia and NAFLD are not causally associated with the risk of GSD. This finding was robust and consistent in the various sensitivity analyses including 4 different MR methods, the validation dataset, sex-stratified assessment, and multivariable MR analysis.

Cholesterol, phospholipid, and bile salts are three major lipid components of bile, and cholesterol supersaturation leads to the precipitation of cholesterol monohydrate crystals followed by agglomeration of the crystals into macroscopic stones (32-36). Results from previous observational studies and reviews showed that hyperlipidemia is a risk factor for GSD (37-40), but the association between each blood lipid and GSD is still controversial. Atamanalp *et al.* found that high LDLC levels were associated with high GSD rates but that low HDLC levels were not (39). However, Andreotti *et al.* reported that high levels of TGs and low levels of HDLC were significantly associated with an increased risk of GSD, while LDLC levels were inversely associated with risk of GSD (40). To date, the conclusions of the relevant research have been inconsistent. Given the limitations of these observational studies, these results might have been driven by biases such as unmeasured confounders or reverse causation (21).

Contrary to previous observational studies, Ferkingstad *et al.* used binomial testing and found that lipid serum levels were not in themselves causative factors in gallstone formation (15). Supporting this finding, Stender *et al.* reported that elevated levels of LDLC were not causally associated with an increased risk of GSD in a one-sample MR study that included 3,323 cases of GSD (41). In our study, each type of blood lipid was considered separately,

Table 3 Mendelian randomization estimations showing the effect of lipid profiles on GSD in male

Exposure	Methods	Odds ratio[a]	95% CI		P value	Ph	Q-statistics
			Lower limit	Upper limit			
LDLC	IVW	0.995	0.991	0.998	3.50E−03	8.25E−02	52.9
	MR-Egger	0.994	0.990	0.999	1.82E−02	6.90E−02	52.8
	Weighted median	0.993	0.989	0.998	6.69E−03	–	–
	Simple median	0.991	0.985	0.998	6.91E−03	–	–
	MR-Egger intercept[b]	0.0001	−0.0002	0.0003	7.51E−01	–	–
HDLC	IVW	1.000	0.997	1.004	9.75E−01	4.05E−02	78.1
	MR-Egger	0.998	0.992	1.004	4.53E−01	4.14E−02	76.8
	Weighted median	1.000	0.995	1.005	9.16E−01	–	–
	Simple median	1.000	0.995	1.006	9.62E−01	–	–
	MR-Egger intercept[b]	0.0001	−0.0001	0.0004	3.34E−01	–	–
Triglycerides	IVW	1.000	0.997	1.004	9.72E−01	4.47E−01	34.4
	MR-Egger	0.998	0.993	1.003	4.67E−01	4.48E−01	33.4
	Weighted median	1.000	0.995	1.006	8.63E−01	–	–
	Simple median	1.003	0.996	1.010	3.60E−01	–	–
	MR-Egger intercept[b]	0.0001	−0.0001	0.0004	3.18E−01	–	–

[a], odds ratio per 1 SD increase; [b], regression coefficient (95% CI). GSD, gallstone disease; CI, confidence interval; LDLC, low-density lipoprotein cholesterol; HDLC, high-density lipoprotein cholesterol; Ph, P value for heterogeneity; SD, standard deviation; IVW, inverse variance-weighting; MR, Mendelian randomization.

Table 4 Mendelian randomization estimations showing the effect of lipid profiles on GSD in female

Exposure	Methods	Odds ratio[a]	95% CI		P value	Ph	Q-statistics
			Lower limit	Upper limit			
LDLC	IVW	0.995	0.990	0.999	9.91E−03	6.95E−02	53.9
	MR-Egger	0.995	0.989	1.000	5.66E−02	5.63E−02	53.9
	Weighted median	0.996	0.991	1.001	9.88E−02	–	–
	Simple median	0.992	0.985	0.999	1.72E−02	–	–
	MR-Egger intercept[b]	0.0002	−0.0004	0.0004	9.79E−01	–	–
HDLC	IVW	0.999	0.994	1.003	5.57E−01	2.48E−02	81.0
	MR-Egger	0.996	0.988	1.005	4.26E−01	2.19E−02	80.5
	Weighted median	0.996	0.99	1.003	2.81E−01	–	–
	Simple median	0.997	0.99	1.004	4.67E−01	–	–
	MR-Egger intercept[b]	0.0002	−0.0003	0.0006	5.67E−01	–	–
Triglycerides	IVW	0.996	0.990	1.001	1.06E−01	2.56E−01	39.0
	MR-Egger	0.992	0.983	1.000	7.30E−02	2.66E−01	37.6
	Weighted median	0.993	0.984	1.001	8.80E−02	–	–
	Simple median	0.998	0.989	1.008	7.45E−01	–	–
	MR-Egger intercept[b]	0.0002	−0.0002	0.0007	2.84E−01	–	–

[a], odds ratio per 1 SD increase; [b], regression coefficient (95% CI). GSD, gallstone disease; CI, confidence interval; LDLC, low-density lipoprotein cholesterol; HDLC, high-density lipoprotein cholesterol; Ph, P value for heterogeneity; SD, standard deviation; IVW, inverse variance-weighting; MR, Mendelian randomization.

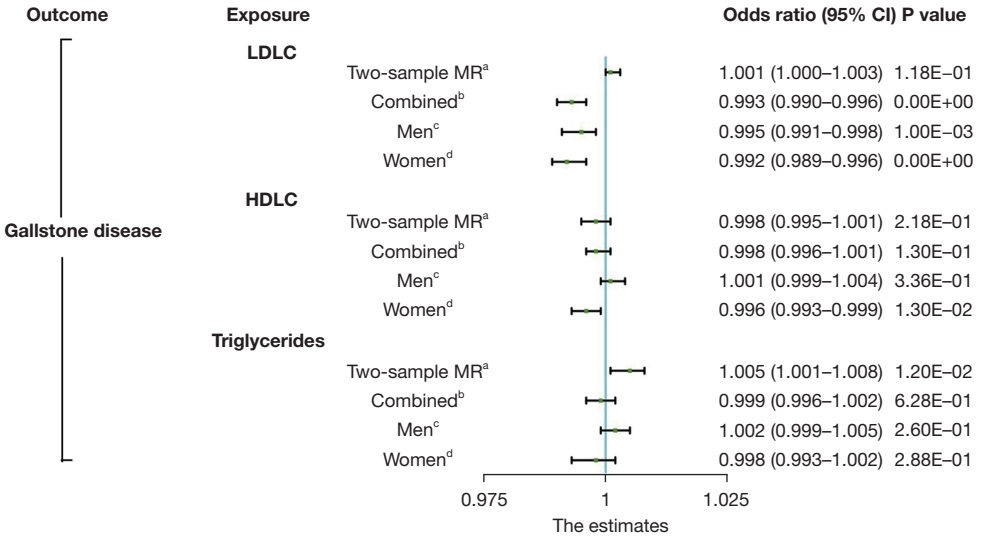

Figure 2 Comparison of the direct causal estimates between plasma lipids and gallstone disease risk via multivariable Mendelian randomization. [a], two-sample MR analysis of the Global Lipids Genetics Consortium study and the UK Biobank cohort; [b], one-sample MR analysis of all participants in the UK Biobank cohort; [c], one-sample MR analysis of male participants in the UK Biobank cohort; [d], one-sample MR analysis of female participants in the UK Biobank cohort. CI, confidence interval; LDLC, low-density lipoprotein cholesterol; HDLC, high-density lipoprotein cholesterol; MR, Mendelian randomization.

and hence our MR analysis had a higher power to confirm that there was no causal association between hyperlipidemia and GSD.

Hepatic steatosis and GSD are commonly found to coexist (19,42-44), and NAFLD and its severity have been independently associated with an increase in GSD (45). However, previous studies were observational investigations, and it has been difficult to perform randomized controlled trials for NAFLD and GSD. It therefore remains unclear whether there is a causal association between NAFLD and GSD. Aside from this, our MR study detected no causal association between genetically driven hepatic steatosis or histologic NAFLD and GSD.

One of the key strengths of our study is that it included 2 very large GWASs with more than 700,000 participants, helping to overcome the power limitations of MR analysis and facilitate the application of several analytical approaches. MR studies are also more robust against confounding than are traditional observational studies because an individual's genetically determined risk for a given condition is fixed throughout their lifetime. Since MR analysis has a high assumption level (46,47), we performed sensitivity analyses, heterogeneity testing, and pleiotropy testing, all of which supported the main findings. To avoid weak instrument bias, we only selected SNPs strongly associated with exposure, and the *F* score values were all larger than 10 for each instrument variable.

In conclusion, this MR study indicates that genetically predicted lipid profiles are not causally associated with GSD in and of themselves. However, like many other MR analyses, this study has several limitations. First, although our findings with respect to the effect of hyperlipidemia and NAFLD on GSD are consistent in TSMR and one-sample MR analyses, the instrument variables only explain approximately 3–8% of the variance of exposure, and thus this study might have been underpowered to detect medium to small effects. Second, with the use of publicly available summary-level GWAS data, we only stratified analyses by sex and were unable to stratify analyses by other covariates of interest such as age, body mass index, and sex hormones. Finally, by using the GLGC study and UK Biobank cohort the majority of participants in our research were of European ancestry, and we were therefore unable to investigate the relationship between lipid profiles and GSD in Asian and African populations.

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Footnote

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Table S1 Harmonized dataset of two-sample Mendelian randomization for the effect of HDLC on gallstone disease

SNP	Effect_allele.HDLC	Other_allele.HDLC	Effect_allele.cholelithiasis	Other_allele.cholelithiasis	Beta.HDLC.GLGC	se.HDLC.GLGC	Beta.HDLC.UK.both_sex	se.HDLC.UK.both_sex	Beta.cholelithiasis.UK.both_sex	se.cholelithiasis.UK.both_sex	Beta.HDLC.UK.female	se.HDLC.UK.female	Beta.cholelithiasis.UK.female	se.cholelithiasis.UK.female	Beta.HDLC.UK.male	se.HDLC.UK.male	Beta.cholelithiasis.UK.male	se.cholelithiasis.UK.male
rs10019888	A	G	A	G	0.027	0.0046	−0.024569	0.0030816	−7.26E−05	0.000544259	−0.034715	0.0046554	−9.61E−05	0.000857473	−0.018554	0.0049851	−5.51E−05	0.00062658
rs10087900	G	A	G	A	0.0231	0.0036	−0.019849	0.002288	−0.000521343	0.000404392	−0.028323	0.0034534	−0.000476448	0.00063636	−0.014819	0.0037047	−0.000583383	0.00046619
rs10282707	C	T	C	T	0.025	0.0035	−0.027124	0.0023284	0.000726787	0.000411672	−0.029358	0.0035179	0.000564464	0.000648459	−0.030678	0.0037662	0.000914013	0.000474048
rs103294	T	C	T	C	0.0523	0.0044	0.041711	0.0027161	0.000948317	0.000479954	0.050161	0.0040937	0.00100196	0.000754337	0.041499	0.0044055	0.000873819	0.000554113
rs10468017	T	C	T	C	0.1179	0.0038	0.10838	0.0024851	−0.000885099	0.000440427	0.1176	0.0037541	−0.00115257	0.000693909	0.12259	0.0040201	−0.000569362	0.000507018
rs10808546	T	C	T	C	0.0409	0.0034	0.034949	0.0022974	0.000877439	0.000406122	0.035385	0.0034668	0.000957038	0.000639104	0.041944	0.0037211	0.000774489	0.000468161
rs11045163	G	A	G	A	0.0217	0.0035	0.019082	0.0023038	0.00049067	0.000407293	0.021237	0.0034735	0.000414264	0.000640736	0.020678	0.0037351	0.000592278	0.000469697
rs11065987	A	G	A	G	0.0222	0.0035	−0.022139	0.002311	0.000583678	0.000408387	−0.022962	0.0034865	0.000262719	0.000642275	−0.026431	0.0037444	0.000964345	0.000471112
rs11789603	T	C	T	C	0.06	0.006	0.069245	0.0036787	0.000169102	0.000650385	0.074592	0.0055648	−0.000119464	0.00102518	0.078176	0.0059425	0.000495132	0.000748366
rs12286037	C	T	C	T	0.1052	0.007	−0.09094	0.0045902	0.000362853	0.000811717	−0.081006	0.0069235	0.000214479	0.00127631	−0.12243	0.0074384	0.000572421	0.000936652
rs12328675	C	T	C	T	0.0447	0.0052	0.039477	0.0035058	−0.000341362	0.000619658	0.048538	0.0053081	0.000204386	0.000978539	0.038179	0.0056568	−0.000976654	0.000711461
rs12412743	C	T	C	T	0.0291	0.0045	−0.026458	0.0030509	6.13E−05	0.000539486	−0.034187	0.0046152	0.000410352	0.000850685	−0.023903	0.0049279	−0.000311944	0.000620473
rs12740374	T	G	T	G	0.0343	0.0041	0.031133	0.0027407	0.00050474	0.000483859	0.033235	0.0041444	0.000855391	0.000762596	0.035728	0.0044286	7.67E−05	0.000556804
rs12748152	C	T	C	T	0.0506	0.0062	−0.040653	0.0041699	0.00032059	0.000736893	−0.050556	0.0062884	0.000273497	0.00115858	−0.038543	0.0067591	0.000358281	0.000850382
rs13099479	A	G	A	G	0.036	0.0062	0.027768	0.0040427	−0.0012673	0.00071498	0.036523	0.0061202	−0.00104722	0.00112844	0.02484	0.0065238	−0.00154065	0.000821416
rs13107325	C	T	C	T	0.0708	0.0078	−0.08196	0.0043295	0.000669971	0.000765675	−0.081643	0.0065678	−0.000130821	0.00121172	−0.10073	0.0069709	0.00159418	0.000876959
rs1482852	G	A	G	A	0.0209	0.0035	0.018488	0.0023328	0.00114663	0.000412419	0.012902	0.0035209	0.000958583	0.000649202	0.029087	0.0037776	0.00136305	0.000475268
rs16842	T	C	T	C	0.03	0.0038	−0.02903	0.0025266	0.000158238	0.000446445	−0.034979	0.003815	0.000450548	0.000703229	−0.029064	0.0040898	−0.000172772	0.000514093
rs1689797	C	A	C	A	0.0358	0.0036	−0.024297	0.0024173	−0.000176758	0.000427003	−0.021991	0.0036485	−0.000449507	0.000672083	−0.032397	0.0039143	0.000147899	0.000492131
rs16942887	A	G	A	G	0.0831	0.0051	0.060936	0.0035695	−0.000211923	0.000630442	0.069773	0.0053997	−0.00066389	0.000993873	0.064501	0.0057656	0.000332643	0.000725292
rs16965220	A	C	A	C	0.0219	0.0037	0.01851	0.0024479	0.000530008	0.000432728	0.019179	0.003695	−0.000116729	0.000681441	0.021828	0.0039635	0.00129667	0.000498438
rs17145738	T	C	T	C	0.0408	0.0053	0.037786	0.0034615	0.00183169	0.000611375	0.045269	0.0052179	0.00215792	0.000960796	0.037734	0.0056136	0.00144035	0.000705901
rs181360	T	G	T	G	0.0376	0.0042	−0.030627	0.0028903	0.000426229	0.000510955	−0.031452	0.0043591	0.00050944	0.000803646	−0.036758	0.0046844	0.000332851	0.000589384
rs1883025	C	T	C	T	0.0698	0.0041	−0.066763	0.0026115	−0.000343792	0.000462011	−0.085794	0.0039423	0.000355303	0.000727619	−0.060307	0.004228	−0.00114054	0.000532121
rs1942880	C	T	C	T	0.0228	0.0036	−0.019857	0.0024309	0.000383128	0.000429707	−0.023263	0.0036668	0.00107381	0.00067633	−0.020652	0.0039391	−0.000415617	0.000495264
rs1980493	T	C	T	C	0.0318	0.0048	−0.030597	0.0030163	0.00134628	0.000533097	−0.037561	0.0045502	0.00206857	0.000839243	−0.029706	0.0048874	0.000500151	0.000614288
rs205262	A	G	A	G	0.0283	0.0039	−0.025857	0.002576	0.000603569	0.000455124	−0.02646	0.0038919	0.000700644	0.000717179	−0.030991	0.0041667	0.000498031	0.000523845
rs2066714	C	T	C	T	0.0453	0.0071	0.045944	0.0034121	−0.000754509	0.000602628	0.053137	0.0051575	−0.00105045	0.00094968	0.048347	0.0055162	−0.000404148	0.000693557
rs2075650	A	G	A	G	0.0554	0.0051	−0.058663	0.0032188	−0.00123164	0.000569215	−0.053986	0.0048611	−0.00176557	0.000895899	−0.077482	0.0052088	−0.000595596	0.000656082
rs2240327	G	A	G	A	0.0242	0.0034	0.022584	0.0022778	−0.000693846	0.000402631	0.026354	0.0034389	−0.00103555	0.000633921	0.023404	0.0036874	−0.000284464	0.000463894
rs2241770	T	C	T	C	0.0989	0.0057	−0.11608	0.0038461	−0.000152056	0.000680742	−0.12643	0.0058067	−0.000292793	0.00107052	−0.13048	0.0062261	−1.75E−05	0.000785389
rs2292101	C	T	C	T	0.0518	0.0096	−0.046017	0.006527	0.00119825	0.00115158	−0.048302	0.0097775	0.00040124	0.00179869	−0.053901	0.010663	0.00213082	0.00133933
rs2303975	A	G	A	G	0.0279	0.0049	0.018523	0.003621	0.000992346	0.000640483	0.032715	0.0054674	0.00139052	0.0010088	0.0069165	0.0058609	0.000515194	0.000737578
rs231492	G	T	G	T	0.0433	0.0077	−0.0551	0.0041614	0.000707353	0.000735905	−0.048309	0.0062996	0.00195729	0.00116086	−0.075183	0.0067162	−0.000729179	0.00084599
rs2454722	G	A	G	A	0.0351	0.0044	0.038045	0.0029597	0.000237565	0.000523185	0.046059	0.0044771	0.000178431	0.000825038	0.037514	0.0047809	0.000296971	0.000601693
rs3741414	T	C	T	C	0.0296	0.004	0.031959	0.0026476	7.79E−05	0.000468387	0.03974	0.0039909	0.000596539	0.000736106	0.030386	0.0042939	−0.000534339	0.000540798

Table S1 *(continued)*

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Table S1 *(continued)*

SNP	Effect_allele.HDLC	Other_allele.HDLC	Effect_allele.cholelithiasis	Other_allele.cholelithiasis	Beta.HDLC.GLGC	se.HDLC.GLGC	Beta.HDLC.UK.both_sex	se.HDLC.UK.both_sex	Beta.cholelithiasis.UK.both_sex	se.cholelithiasis.UK.both_sex	Beta.HDLC.UK.female	se.HDLC.UK.female	Beta.cholelithiasis.UK.female	se.cholelithiasis.UK.female	Beta.HDLC.UK.male	se.HDLC.UK.male	Beta.cholelithiasis.UK.male	se.cholelithiasis.UK.male
rs3822072	G	A	G	A	0.0251	0.0034	−0.020892	0.0022873	−0.000547031	0.000404405	−0.032495	0.0034498	−0.000753746	0.000636198	−0.012518	0.0037068	−0.000309604	0.000466362
rs3936511	A	G	A	G	0.0308	0.0046	−0.032082	0.0029097	1.49E−05	0.000514443	−0.036609	0.0043998	0.000816183	0.000810473	−0.034176	0.0047019	−0.000909622	0.000592267
rs3996352	G	A	G	A	0.0296	0.0034	0.031122	0.0022795	−0.000192975	0.000402853	0.047545	0.0034368	−0.00020572	0.000633745	0.019497	0.0036955	−0.000184879	0.000464588
rs4148005	T	G	T	G	0.0283	0.0036	−0.019184	0.0024502	0.000435196	0.000432995	−0.01781	0.0036933	0.000209067	0.00068067	−0.024835	0.003974	0.000679715	0.000499772
rs4379922	C	T	C	T	0.0247	0.0036	0.023015	0.002354	0.000162479	0.000416097	0.026514	0.0035527	0.000424889	0.000655176	0.024443	0.0038123	−0.000133338	0.000479361
rs4465830	A	G	A	G	0.0597	0.0044	−0.063308	0.0029151	−3.90E−05	0.000515737	−0.089434	0.0044006	−0.000913932	0.000811933	−0.04797	0.0047193	0.00097863	0.000594252
rs4660293	A	G	A	G	0.0353	0.004	−0.042216	0.0026858	0.000535331	0.000475122	−0.044915	0.0040525	0.00083609	0.000747465	−0.048655	0.004351	0.00019015	0.000547905
rs4693156	C	T	C	T	0.0197	0.0035	0.018287	0.0023456	0.000672762	0.000414675	0.01854	0.0035355	0.00109012	0.000651904	0.021868	0.0038044	0.000209851	0.000478607
rs4917014	G	T	G	T	0.0222	0.0036	0.015987	0.0024464	0.000571274	0.000432391	0.023043	0.0036929	0.000711575	0.000680578	0.011132	0.0039611	0.000393549	0.000498339
rs492571	T	C	T	C	0.0663	0.009	−0.041743	0.0057213	0.00165069	0.00101379	−0.055287	0.0086328	0.00250382	0.00159507	−0.035456	0.0092682	0.000686533	0.00116901
rs4976033	A	G	A	G	0.0215	0.0037	−0.012458	0.0023529	3.14E−05	0.000415844	−0.013915	0.0035493	−8.81E−05	0.000654178	−0.013889	0.0038128	0.000164054	0.000479573
rs499974	C	A	C	A	0.0263	0.0044	−0.034754	0.0031368	0.000300973	0.00055427	−0.041732	0.0047234	0.00139133	0.000870287	−0.034605	0.005093	−0.000991572	0.00064061
rs593245	T	C	T	C	0.0208	0.0038	0.01161	0.0022914	−0.000774433	0.000404684	0.010439	0.0034596	−0.00134566	0.000636862	0.015217	0.0037093	−0.000108883	0.000466501
rs6031587	C	T	C	T	0.0488	0.0074	−0.053726	0.0044944	0.0024202	0.000794043	−0.055242	0.0067878	0.00323551	0.00125154	−0.063941	0.007273	0.00150084	0.000913702
rs633695	G	A	G	A	0.0885	0.0054	0.08496	0.0025125	0.00018662	0.000444804	0.096275	0.0037915	−6.37E−05	0.000700218	0.09127	0.0040694	0.000460859	0.000512553
rs676210	A	G	A	G	0.066	0.004	0.062039	0.0028222	0.00100913	0.000498922	0.069442	0.0042593	0.00184087	0.000785546	0.067623	0.0045704	3.36E−05	0.000574798
rs6898870	G	A	G	A	0.0298	0.0055	−0.01429	0.0025919	0.000240486	0.000458097	−0.0095124	0.0039115	0.000688626	0.000721016	−0.022652	0.004198	−0.000262966	0.000527988
rs7306660	G	A	G	A	0.0345	0.0036	−0.024604	0.0023791	−0.000182522	0.000420403	−0.031831	0.0035973	−0.000482376	0.000662445	−0.022059	0.0038447	0.000169924	0.000483891
rs737337	T	C	T	C	0.0565	0.0061	−0.054572	0.0042869	0.000133182	0.000757782	−0.055705	0.0064962	0.000495735	0.001197	−0.065365	0.006911	−0.000313668	0.000869817
rs765548	T	C	T	C	0.1065	0.0038	0.11913	0.0025771	−0.000526262	0.000456974	0.11678	0.0038985	−0.00096185	0.000719632	0.14903	0.0041619	−5.01E−05	0.000526385
rs884366	G	A	G	A	0.0199	0.0037	−0.018314	0.002464	−0.000461024	0.000435496	−0.024642	0.0037191	−0.000617263	0.000685308	−0.014953	0.00399	−0.000273676	0.000502051
rs9457931	A	G	A	G	0.0552	0.0073	−0.030468	0.0048529	−0.00103053	0.000857578	−0.02809	0.0073404	−0.00214183	0.00135002	−0.040419	0.0078393	0.000236768	0.000988209
rs998584	C	A	C	A	0.026	0.0038	−0.033179	0.0022835	5.98E−05	0.000403569	−0.034788	0.0034501	−0.000359708	0.000635668	−0.039329	0.0036933	0.000559874	0.00046473

Female: women specify participants in the UK Biobank; male: men specify participants in the UK Biobank. Beta, regression coefficient; se, standard error; HDLC, high-density lipoprotein cholesterol; both_sex, combined sex participants in the UK Biobank; GLGC, Global Lipids Genetics Consortium; SNP, single-nucleotide polymorphism.

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Table S2 Harmonized dataset of two-sample Mendelian randomization for the effect of triglycerides on gallstone disease

SNP	Effect_ allele.TG	Other_ allele.TG	Effect_allele. cholelithiasis	Other_allele. cholelithiasis	Beta. TG.GLGC	se.TG. GLGC	Beta. TG.UK. both_sex	se.TG. UK.both_ sex	Beta. cholelithiasis. UK.both_sex	se.cholelithiasis. UK.both_sex	beta. TG.UK. female	se.TG. UK.female	Beta. cholelithiasis. UK.female	se.cholelithiasis. UK.female	Beta.TG.UK. male	se.TG. UK.male	Beta. cholelithiasis. UK.male	se.cholelithiasis. UK.male
rs10401969	T	C	T	C	0.121	0.0065	−0.099379	0.004362	0.000531676	0.000757307	−0.081062	0.0059918	0.000677146	0.00119054	−0.12459	0.0066721	0.000352387	0.000874068
rs10501321	T	C	T	C	0.0216	0.0035	−0.022928	0.0024759	−0.000163473	0.000429506	−0.01843	0.0034044	−0.000667665	0.000676072	−0.028831	0.0037829	0.000420815	0.000494989
rs10513688	A	G	A	G	0.0306	0.0056	0.026017	0.0039316	0.000435703	0.000681974	0.030092	0.0053987	0.00128954	0.0010726	0.022901	0.0060162	−0.000566674	0.000786683
rs10861661	C	A	C	A	0.0227	0.0041	0.017942	0.0026883	−0.000534271	0.000465992	0.018454	0.0037052	−0.000934354	0.000735365	0.018255	0.0040961	−7.43E−05	0.000535473
rs11057408	G	T	G	T	0.0258	0.0035	−0.027928	0.0024585	0.00080572	0.000426334	−0.036305	0.0033829	0.000819368	0.000671797	−0.019988	0.0037529	0.000782971	0.000490722
rs11613352	C	T	C	T	0.028	0.0039	−0.028067	0.0027009	3.94E−05	0.000468681	−0.024369	0.0037081	0.000595823	0.000736497	−0.033686	0.0041339	−0.000616153	0.000541195
rs11820504	C	T	C	T	0.0604	0.0044	0.067505	0.0030573	−0.000344427	0.000530629	0.067433	0.0042069	−0.00124828	0.000836081	0.071214	0.0046671	0.00070735	0.000610834
rs1211644	T	C	T	C	0.0298	0.0053	−0.018736	0.0026331	−0.000367478	0.000456761	−0.022972	0.0036146	9.34E−05	0.000717743	−0.014913	0.0040309	−0.000896656	0.000527453
rs12412743	T	C	T	C	0.0238	0.0044	0.016102	0.0031102	6.13E−05	0.000539486	0.020078	0.004284	0.000410352	0.000850685	0.012433	0.0047423	−0.000311944	0.000620473
rs12602912	T	C	T	C	0.0241	0.0041	0.024129	0.0029281	0.000861337	0.000507722	0.02429	0.0040251	0.00114964	0.000798995	0.025239	0.004475	0.000526359	0.000585278
rs12676857	C	T	C	T	0.0332	0.0046	0.034976	0.0032805	−0.000191865	0.000569072	0.034155	0.0045156	−0.000860148	0.000896461	0.037763	0.005006	0.000592019	0.000655234
rs12679834	T	C	T	C	0.1647	0.0054	−0.19636	0.0038337	−0.000528781	0.00066743	−0.19469	0.0052721	−0.00042999	0.00105041	−0.20839	0.005856	−0.00065831	0.00076932
rs12748152	T	C	T	C	0.0372	0.0059	0.03194	0.0042467	0.00032059	0.000736893	0.038854	0.0058283	0.000273497	0.00115858	0.025615	0.0065025	0.000358281	0.000850382
rs13389219	C	T	C	T	0.0271	0.0034	−0.036997	0.0023726	−0.000116974	0.000411854	−0.04787	0.0032599	−0.000536492	0.00064811	−0.026814	0.0036278	0.000361418	0.000474785
rs17513135	T	C	T	C	0.022	0.0039	0.024565	0.0027687	0.000510905	0.000480194	0.026677	0.0038053	0.00083464	0.000755593	0.023434	0.0042321	0.000143022	0.000553625
rs1883025	C	T	C	T	0.0219	0.004	−0.018986	0.0026639	−0.000343792	0.000462011	−0.020669	0.0036642	0.000355303	0.000727619	−0.018101	0.0040685	−0.00114054	0.000532121
rs2068888	G	A	G	A	0.0241	0.0034	−0.030814	0.0023294	−0.00025375	0.0004042	−0.029817	0.0032021	−0.000834351	0.000636154	−0.033404	0.00356	0.000408101	0.0004659
rs2251830	C	A	C	A	0.0236	0.0036	−0.015452	0.0023336	0.000589998	0.000404861	−0.013688	0.0032093	0.0012169	0.000637593	−0.01801	0.0035647	−0.000133357	0.000466312
rs2954022	C	A	C	A	0.078	0.0033	−0.090137	0.0023214	0.000900101	0.000403401	−0.084837	0.0031927	0.00114037	0.00063514	−0.10078	0.0035455	0.000608082	0.000464755
rs3198697	C	T	C	T	0.0198	0.0034	−0.024684	0.0023557	−0.000241451	0.00040874	−0.024553	0.0032349	−0.000751999	0.00064245	−0.026186	0.0036047	0.000359952	0.000471847
rs3760627	C	T	C	T	0.0189	0.0034	0.017197	0.0023288	0.000233052	0.00040373	0.019613	0.0031986	−3.11E−05	0.000634864	0.0154	0.0035628	0.000551836	0.000465839
rs4587594	G	A	G	A	0.0694	0.0035	−0.080107	0.0024238	0.000102297	0.000421031	−0.073549	0.0033358	0.000441091	0.000663321	−0.091551	0.0036991	−0.000297538	0.000484708
rs4719841	G	A	G	A	0.0232	0.0034	0.025629	0.0025073	0.000594046	0.000435049	0.028861	0.0034524	0.000963709	0.000685656	0.023324	0.0038244	0.000169229	0.000500641
rs4804311	A	G	A	G	0.0392	0.006	−0.046466	0.0042414	−8.35E−05	0.000735884	−0.046614	0.0058369	0.000520112	0.0011595	−0.048459	0.0064737	−0.000761766	0.000847068
rs492571	C	T	C	T	0.0799	0.0088	0.073756	0.0058373	0.00165069	0.00101379	0.084051	0.0080265	0.00250382	0.00159507	0.066444	0.0089185	0.000686533	0.00116901
rs676210	G	A	G	A	0.0733	0.0039	−0.076578	0.0028741	0.00100913	0.000498922	−0.081252	0.0039517	0.00184087	0.000785546	−0.07514	0.0043912	3.36E−05	0.000574798
rs6831256	G	A	G	A	0.0258	0.0035	0.023806	0.0023498	6.48E−05	0.000407496	0.028335	0.0032282	−0.00049967	0.000640974	0.019968	0.0035935	0.000731536	0.000469998
rs6995541	G	A	G	A	0.0265	0.0037	0.025804	0.0025704	−0.000214148	0.000445768	0.02417	0.0035297	−0.000275463	0.000700783	0.028887	0.003933	−0.000144023	0.000514479
rs714052	A	G	A	G	0.1084	0.005	−0.1244	0.0035021	0.00153531	0.000608615	−0.13325	0.0048061	0.00200846	0.000956506	−0.12092	0.0053626	0.000969708	0.000702674
rs7205804	G	A	G	A	0.0367	0.0034	−0.03117	0.0023377	3.35E−06	0.000405544	−0.028914	0.0032099	−0.0004044	0.000637442	−0.035323	0.0035774	0.000483926	0.000468141
rs72555385	G	A	G	A	0.0749	0.0124	0.066648	0.0054047	1.50E−05	0.000936889	0.068328	0.0074473	0.000395581	0.0014783	0.068451	0.0082374	−0.000427318	0.00107671
rs749671	G	A	G	A	0.0211	0.0034	−0.014732	0.0024009	−0.000561806	0.000416539	−0.021209	0.0032991	−0.000570191	0.000655261	−0.0082727	0.0036709	−0.000550313	0.000480381
rs8077889	C	A	C	A	0.0252	0.0042	0.017051	0.0028315	8.93E−05	0.000491272	0.017457	0.0038934	0.000344642	0.00077296	0.017435	0.0043261	−0.000204843	0.000566464
rs9686661	T	C	T	C	0.0379	0.0044	0.044983	0.0029181	−0.000227378	0.000506298	0.054406	0.0040172	0.000319563	0.000797755	0.036792	0.004452	−0.0008637	0.000582803
rs998584	A	C	A	C	0.0293	0.0037	0.03985	0.0023261	5.98E−05	0.000403569	0.042217	0.0032007	−0.000359708	0.000635668	0.039566	0.0035507	0.000559874	0.00046473

Female: women specify participants in the UK Biobank; male: men specify participants in the UK Biobank. Beta, regression coefficient; se, standard error; TG, triglycerides; both_sex, combined sex participants in the UK Biobank; GLGC, Global Lipids Genetics Consortium; SNP, single-nucleotide polymorphism.

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Table S3 Harmonized dataset of two-sample Mendelian randomization for the effect of liver fat content on gallstone disease

SNP	Effect_allele.NAFLD	Other_allele.NAFLD	Effect_allele.cholelithiasis	Other_allele.cholelithiasis	Beta.Hep_steatosis.GOLD	se.Hep_steatosis.GOLD	Beta.NAFLD.AGES	se.NAFLD.AGES	Beta.cholelithiasis.UK	se.cholelithiasis.UK
rs2228603	T	C	T	C	0.238	0.035	0.184	0.053	0.000486059	0.000761385
rs738409	G	C	G	C	0.261	0.021	0.232	0.032	−0.00220539	0.000488266

Beta, regression coefficient; se, standard error; Hep_steatosis, hepatic steatosis; NAFLD, non-alcoholic fatty liver disease; GOLD, Genetics of Obesity-related Liver Disease; AGES, Old Order Amish, Age, Gene/Environment Susceptibility-Reykjavik study; UK, the UK Biobank cohort; SNP, single-nucleotide polymorphism.

Table S4 Harmonized dataset of two-sample Mendelian randomization for the effect of LDLC on gallstone disease

SNP	Effect_allele.LDLC	Other_allele.LDLC	Effect_allele.cholelithiasis	Other_allele.cholelithiasis	Beta.LDLC.GLGC	se.LDLC.GLGC	Beta.LDLC.UK.both_sex	se.LDLC.UK.both_sex	Beta.cholelithiasis.UK.both_sex	se.cholelithiasis.UK.both_sex	Beta.LDLC.UK.female	se.LDLC.UK.female	Beta.cholelithiasis.UK.female	se.cholelithiasis.UK.female	Beta.LDLC.UK.male	se.LDLC.UK.male	Beta.cholelithiasis.UK.male	se.cholelithiasis.UK.male
rs10195252	T	C	T	C	0.0238	0.0039	−0.013394	0.0023987	−0.000272351	0.000410046	−0.020526	0.0032665	−0.000676653	0.00064565	−0.0054446	0.0035642	0.000187234	0.000472377
rs10947332	A	G	A	G	0.0504	0.0056	0.045433	0.0036141	−0.00103338	0.000618097	0.052541	0.0049035	−0.00162052	0.000969445	0.037786	0.0053934	−0.000339619	0.000715308
rs11065987	A	G	A	G	0.0269	0.0038	−0.025275	0.0023896	0.000583678	0.000408387	−0.023866	0.0032515	0.000262719	0.000642275	−0.027153	0.0035541	0.000964345	0.000471112
rs112201728	T	C	T	C	0.0675	0.0104	0.054991	0.0046338	−0.00049049	0.000791708	0.070652	0.0062946	−0.000968118	0.00124287	0.037466	0.0069049	9.24E−05	0.000915277
rs11220462	A	G	A	G	0.059	0.0059	0.040434	0.0034608	0.000382179	0.000591849	0.034022	0.0047092	−0.000150584	0.000930674	0.048587	0.0051467	0.00100583	0.000682857
rs11563251	T	C	T	C	0.0345	0.0062	0.020104	0.0037514	−0.00221903	0.000641197	0.02591	0.0051005	−0.00226225	0.00100781	0.014109	0.0055843	−0.00214888	0.000740229
rs11591147	G	T	G	T	0.497	0.018	−0.34847	0.0088975	0.000179825	0.00152518	−0.35623	0.012134	−0.00168225	0.00240308	−0.34543	0.013199	0.00232818	0.00175572
rs117733303	G	A	G	A	0.1551	0.022	0.08793	0.0087077	−0.00290238	0.00148804	0.11606	0.01186	−0.00579976	0.0023381	0.057819	0.012936	0.000528062	0.00171848
rs12670798	C	T	C	T	0.0344	0.0043	0.027899	0.0027261	−0.000217255	0.00046577	0.033656	0.0037087	−0.000366807	0.000732217	0.021685	0.0040551	−4.48E−05	0.000537575
rs12721109	G	A	G	A	0.4462	0.0183	−0.34087	0.0073581	0.00139244	0.00126199	−0.39975	0.010015	1.42E−05	0.00198811	−0.28031	0.010936	0.00299465	0.00145294
rs12740374	G	T	G	T	0.161	0.0044	−0.1181	0.0028258	0.00050474	0.000483859	−0.11046	0.0038552	0.000855391	0.000762596	−0.1284	0.0041897	7.67E−05	0.000556804
rs12748152	T	C	T	C	0.0499	0.0066	0.011806	0.0043104	0.00032059	0.000736893	0.014833	0.0058594	0.000273497	0.00115858	0.0084685	0.0064182	0.000358281	0.000850382
rs13315871	G	A	G	A	0.0344	0.0063	−0.028708	0.0041849	0.000264737	0.000714333	−0.030069	0.0057161	0.000529921	0.00112772	−0.027495	0.0061965	−4.79E−05	0.000820422
rs1367117	A	G	A	G	0.1186	0.004	0.082043	0.002482	−0.000916447	0.00042493	0.090315	0.0033736	−0.00103018	0.00066779	0.073846	0.0036957	−0.000788718	0.00049064
rs1408272	T	G	T	G	0.052	0.0083	−0.049188	0.0043406	−0.000373132	0.000742093	−0.047485	0.0059054	−0.00111693	0.00116646	−0.051789	0.0064568	0.000485838	0.000856649
rs1535	A	G	A	G	0.0529	0.0038	−0.031219	0.0024717	0.00154735	0.000422381	−0.034077	0.0033695	0.00234594	0.000665549	−0.028335	0.0036682	0.00059189	0.000486185
rs1564348	C	T	C	T	0.0481	0.005	0.033276	0.0031276	−0.000148865	0.000534696	0.045218	0.0042561	−0.000394528	0.000840901	0.020036	0.004651	0.000142107	0.000616883
rs17508045	T	C	T	C	0.0488	0.0066	−0.034993	0.0041561	−0.000161859	0.000710633	−0.034394	0.0056778	−0.0012224	0.00112213	−0.03658	0.0061527	0.00105585	0.000815972
rs17789218	T	C	T	C	0.0241	0.0043	−0.017218	0.0027435	0.000874905	0.000468761	−0.018567	0.0037303	0.00102662	0.000736761	−0.015758	0.0040838	0.000681001	0.000541161
rs1883025	C	T	C	T	0.0296	0.0044	−0.023862	0.0027037	−0.000343792	0.000462011	−0.026426	0.003683	0.000355303	0.000727619	−0.021399	0.0040159	−0.00114054	0.000532121
rs2000999	A	G	A	G	0.065	0.0046	0.049659	0.0030082	0.000454092	0.000514194	0.05644	0.0040938	0.000161443	0.000808948	0.042614	0.0044732	0.000786531	0.000592952
rs2228603	C	T	C	T	0.104	0.0072	−0.089614	0.0044515	0.000486059	0.000761385	−0.064558	0.006073	0.00127081	0.00119989	−0.11934	0.0065997	−0.000420847	0.000876243
rs2294261	A	C	A	C	0.0333	0.0037	−0.015909	0.0023592	−1.39E−05	0.000403073	−0.018899	0.0032122	−0.000333387	0.000634297	−0.012747	0.0035061	0.000339558	0.000464675
rs247616	C	T	C	T	0.0547	0.0041	−0.033836	0.002514	−2.34E−05	0.000429705	−0.048311	0.0034232	−0.00018878	0.000676377	−0.017415	0.0037353	0.000185386	0.000495214
rs2737252	G	A	G	A	0.0314	0.0041	−0.02164	0.0026124	−0.000297307	0.000446345	−0.023404	0.0035551	−0.00125157	0.00070213	−0.01971	0.0038845	0.000802905	0.000514755
rs2738459	A	C	A	C	0.0532	0.0058	−0.022332	0.0023663	0.000186694	0.00040444	−0.024775	0.0032208	0.000628625	0.000636198	−0.019809	0.003518	−0.000321547	0.000466456
rs2965157	T	C	T	C	0.1886	0.0112	−0.21328	0.0069524	0.000127013	0.00118835	−0.24636	0.0094533	−0.000234755	0.00186807	−0.17918	0.010347	0.000541374	0.00137164
rs314253	T	C	T	C	0.0242	0.0038	−0.015604	0.0024627	0.000766988	0.000420855	−0.011216	0.0033562	0.000821382	0.00066297	−0.020912	0.0036559	0.000713611	0.000484559
rs3780181	A	G	A	G	0.0445	0.0074	−0.028533	0.0047333	0.000987286	0.000808958	−0.031248	0.0064404	0.00134956	0.00127159	−0.026345	0.00704	0.000538136	0.000933809
rs6016373	A	G	A	G	0.0349	0.0037	−0.025221	0.00244	−2.77E−06	0.00041701	−0.027803	0.0033196	0.000232035	0.000656048	−0.022765	0.0036293	−0.00028023	0.000480878

Table S4 *(continued)*

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Table S4 *(continued)*

SNP	Effect_allele.LDLC	Other_allele.LDLC	Effect_allele.cholelithiasis	Other_allele.cholelithiasis	Beta.LDLC.GLGC	se.LDLC.GLGC	Beta.LDLC.UK.both_sex	se.LDLC.UK.both_sex	Beta.cholelithiasis.UK.both_sex	se.cholelithiasis.UK.both_sex	Beta.LDLC.UK.female	se.LDLC.UK.female	Beta.cholelithiasis.UK.female	se.cholelithiasis.UK.female	Beta.LDLC.UK.male	se.LDLC.UK.male	Beta.cholelithiasis.UK.male	se.cholelithiasis.UK.male
rs6065311	C	T	C	T	0.0417	0.0036	0.025124	0.002359	0.000696426	0.000403162	0.032556	0.0032076	0.000362603	0.000633656	0.017005	0.0035111	0.00108216	0.000465439
rs6511720	G	T	G	T	0.2209	0.0061	−0.17743	0.0036249	0.000599792	0.000621574	−0.19144	0.0049372	0.000671822	0.000978832	−0.1636	0.0053846	0.00049627	0.000715964
rs7254892	G	A	G	A	0.4853	0.0119	−0.41887	0.0067697	0.00432116	0.00116263	−0.47472	0.009186	0.00459571	0.00182796	−0.36281	0.010097	0.00399961	0.00134174
rs72902576	T	G	T	G	0.0933	0.0133	−0.078918	0.0057713	0.00167103	0.000986164	−0.069228	0.0078601	0.00254732	0.00155134	−0.090445	0.0085744	0.000638709	0.00113732
rs75687619	T	G	T	G	0.1735	0.0161	0.17049	0.0073867	−0.00209175	0.00126301	0.19452	0.010078	−0.00224586	0.00198751	0.14514	0.010952	−0.00194031	0.00145597
rs7640978	C	T	C	T	0.0392	0.0069	−0.034954	0.0041269	−0.00120938	0.000705535	−0.033924	0.0056148	−0.000792659	0.00110907	−0.036144	0.0061386	−0.00169213	0.000814354
rs7703051	A	C	A	C	0.0727	0.0037	0.060545	0.0024289	0.000143863	0.000415435	0.067811	0.0033086	0.000158891	0.000654207	0.052838	0.0036078	0.000133409	0.000478544
rs7832643	T	G	T	G	0.0339	0.0038	0.014503	0.0024071	0.000220301	0.000411442	0.015922	0.0032755	0.000598461	0.000647207	0.012999	0.0035799	−0.000230316	0.000474536
rs8017377	A	G	A	G	0.0303	0.0038	0.01645	0.0023544	0.000473956	0.000402363	0.019541	0.0032063	0.00101748	0.00063331	0.013177	0.0034984	−0.000138684	0.000463735
rs868943	G	A	G	A	0.0264	0.0037	−0.01454	0.0023801	0.000358961	0.000406662	−0.014653	0.0032438	0.000502691	0.000640549	−0.01473	0.0035334	0.000181818	0.000468303
rs9875338	G	A	G	A	0.027	0.0037	−0.015745	0.0024036	−0.000307445	0.000410629	−0.015721	0.0032718	−0.00072233	0.000646093	−0.016053	0.0035732	0.000181854	0.000473454

Female: women specify participants in the UK Biobank; male: men specify participants in the UK Biobank. Beta, regression coefficient; se, standard error; LDLC, low density lipoprotein cholesterol; both_sex, combined sex participants in the UK Biobank; GLGC, Global Lipids Genetics Consortium; SNP, single-nucleotide polymorphism.

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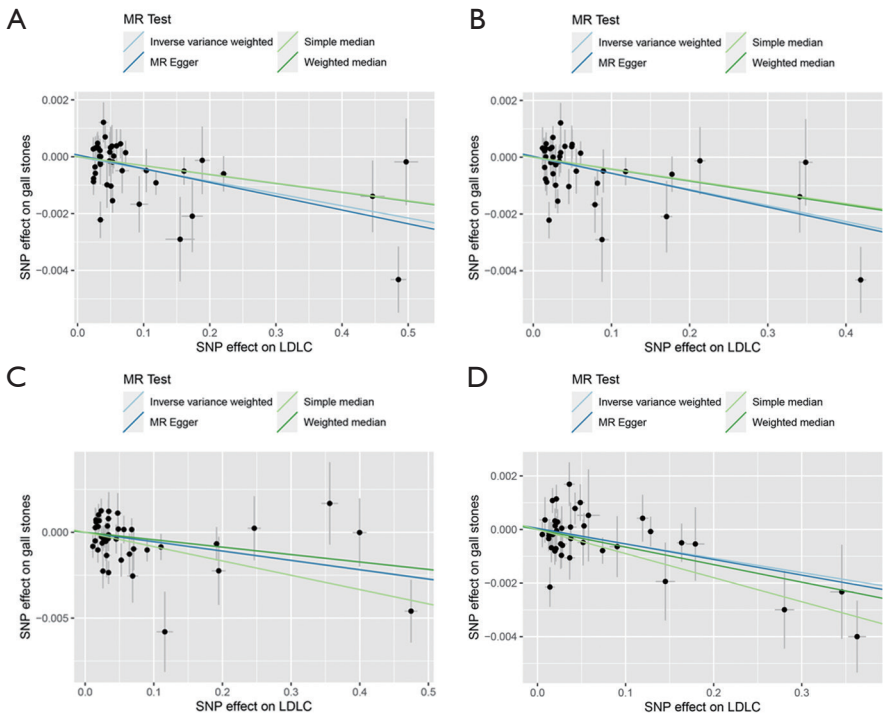

Figure S1 Comparison of the causal estimates between LDLC and gallstone disease from the various MR methods as sensitivity analysis. (A) Comparison of the two-sample MR analysis causal estimates between LDLC and gallstone disease from the Global Lipids Genetics Consortium and the UK Biobank cohort. (B) Comparison of the one-sample MR analysis causal estimates between LDLC and gallstone disease from the UK Biobank cohort. (C) Comparison of the one-sample MR analysis causal estimates between LDLC and gallstone disease from women-specify populations in the UK Biobank cohort. (D) Comparison of the one-sample MR analysis causal estimates between LDLC and gallstone disease from men-specify populations in the UK Biobank cohort. SNP, single-nucleotide polymorphism; MR, Mendelian randomization; LDLC, low-density lipoprotein cholesterol.

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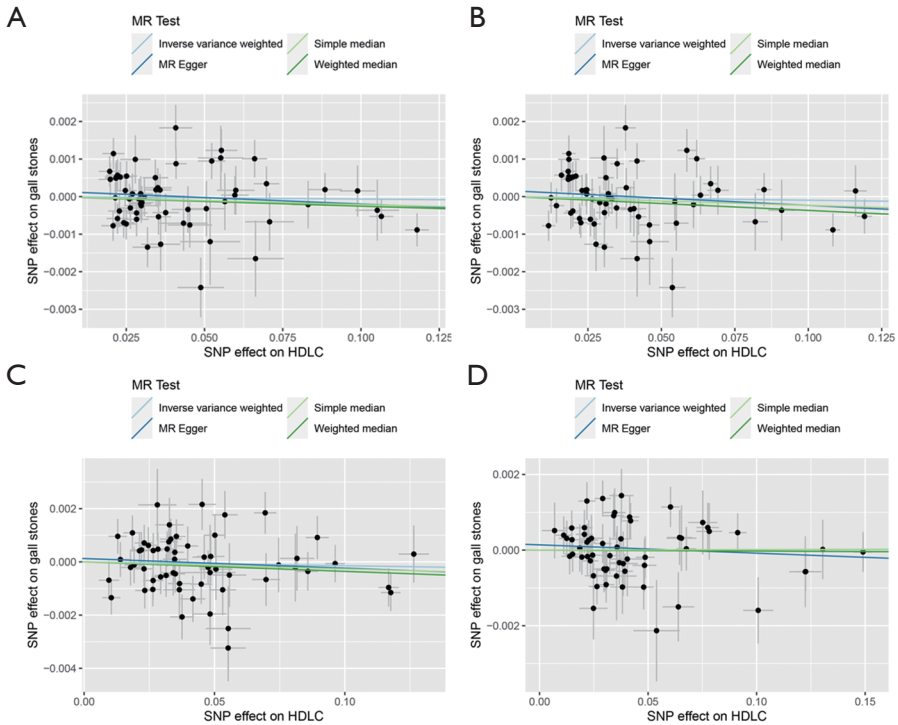

Figure S2 Comparison of the causal estimates between HDLC and gallstone disease from the various MR methods as sensitivity analysis. (A) Comparison of the two-sample MR analysis causal estimates between HDLC and gallstone disease from the Global Lipids Genetics Consortium and the UK Biobank cohort. (B) Comparison of the one-sample MR analysis causal estimates between HDLC and gallstone disease from the UK Biobank cohort. (C) Comparison of the one-sample MR analysis causal estimates between HDLC and gallstone disease from women-specify populations in the UK Biobank cohort. (D) Comparison of the one-sample MR analysis causal estimates between HDLC and gallstone disease from men-specify populations in the UK Biobank cohort. SNP, single-nucleotide polymorphism; MR, Mendelian randomization; HDLC, high-density lipoprotein cholesterol.

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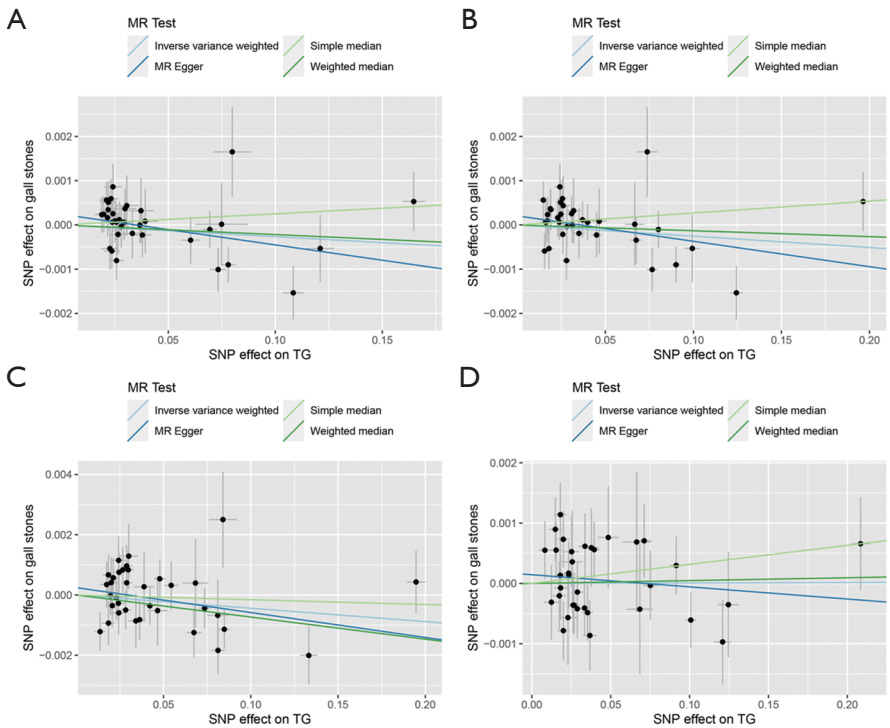

Figure S3 Comparison of the causal estimates between TGs and gallstone disease from the various MR methods as sensitivity analysis. (A) Comparison of the two-sample MR analysis causal estimates between TGs and gallstone disease from the Global Lipids Genetics Consortium and the UK Biobank cohort. (B) Comparison of the one-sample MR analysis causal estimates between TGs and gallstone disease from the UK Biobank cohort. (C) Comparison of the one-sample MR analysis causal estimates between TGs and gallstone disease from women-specify populations in the UK Biobank cohort. (D) Comparison of the one-sample MR analysis causal estimates between TGs and gallstone disease from men-specify populations in the UK Biobank cohort. SNP, single-nucleotide polymorphism; MR, Mendelian randomization; TG, triglyceride.

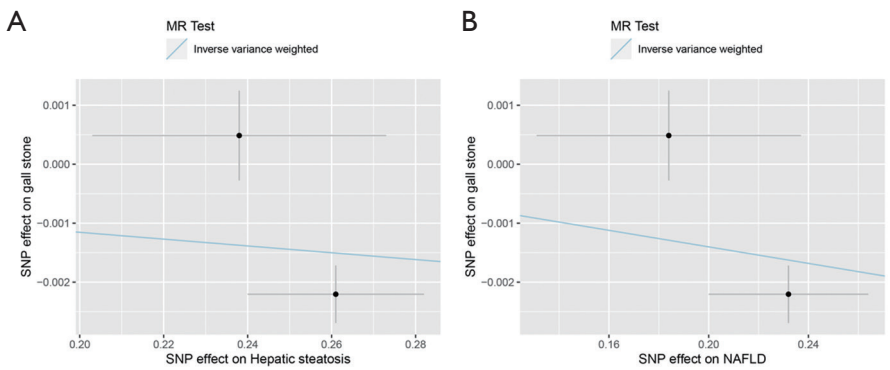

Figure S4 Comparison of the causal estimates between liver fat content and gallstone disease from the various MR methods as sensitivity analysis. (A) Comparison of the two-sample MR analysis causal estimates between hepatic steatosis and gallstone disease from the Genetics of Obesity-related Liver Disease and the UK Biobank cohort. (B) Comparison of the one-sample MR analysis causal estimates between non-alcoholic fatty liver disease and gallstone disease from the Old Order Amish, Age, Gene/Environment Susceptibility-Reykjavik study and the UK Biobank cohort. SNP, single-nucleotide polymorphism; MR, Mendelian randomization; NAFLD, non-alcoholic fatty liver disease.

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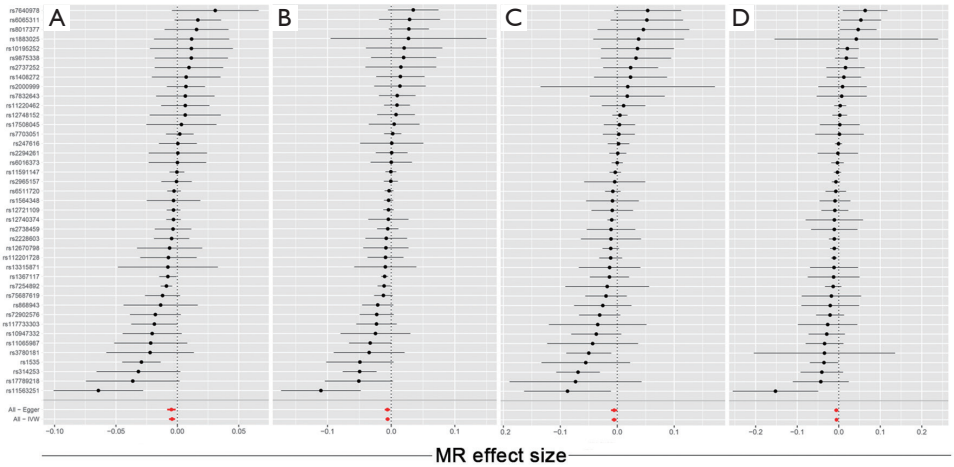

Figure S5 Forest plot of variant specific inverse variance estimates for causal association between LDLC and gallstone disease. (A) Variant specific inverse variance estimates for causal association between LDLC and gallstone disease from the Global Lipids Genetics Consortium and the UK Biobank cohort. (B) Variant specific inverse variance estimates for causal association between LDLC and gallstone disease from the UK Biobank cohort. (C) Variant specific inverse variance estimates for causal association between LDLC and gallstone disease from women-specify populations in the UK Biobank cohort. (D) Variant specific inverse variance estimates for causal association between LDLC and gallstone disease from men-specify populations in the UK Biobank cohort. IVW, inverse variance-weighting; LDLC, low-density lipoprotein cholesterol; MR, Mendelian randomization.

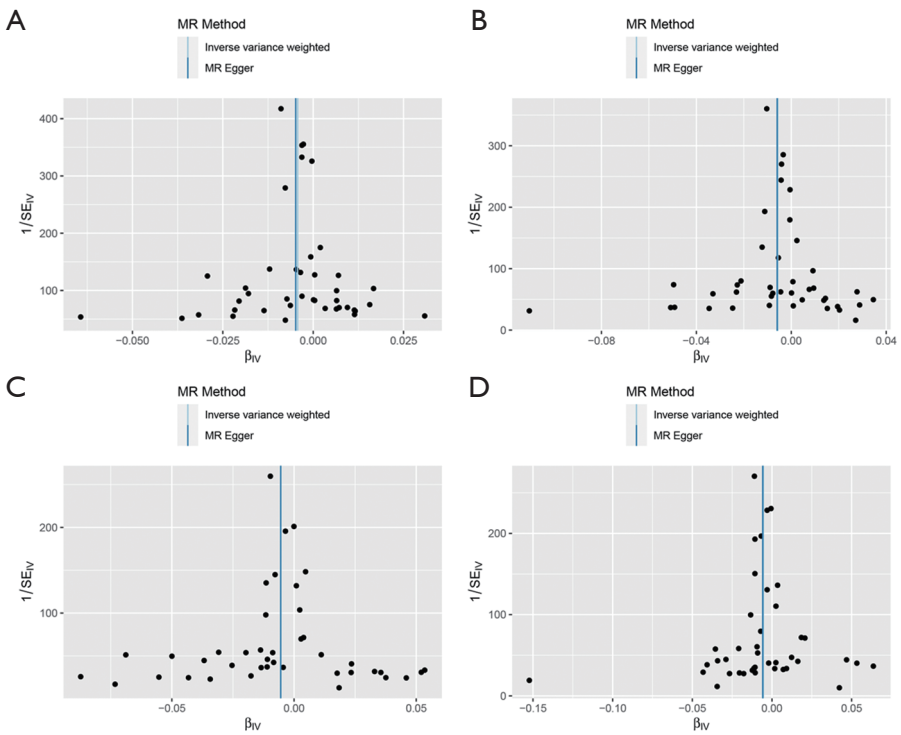

Figure S6 Funnel plot of causal association between LDLC and gallstone disease. (A) Funnel plot for causal association between LDLC and gallstone disease from the Global Lipids Genetics Consortium and the UK Biobank cohort. (B) Funnel plot for causal association between LDLC and gallstone disease from the UK Biobank cohort. (C) Funnel plot for causal association between LDLC and gallstone disease from women-specify populations in the UK Biobank cohort. (D) Funnel plot for causal association between LDLC and gallstone disease from men-specify populations in the UK Biobank cohort. LDLC, low-density lipoprotein cholesterol; MR, Mendelian randomization; SE, standard error.

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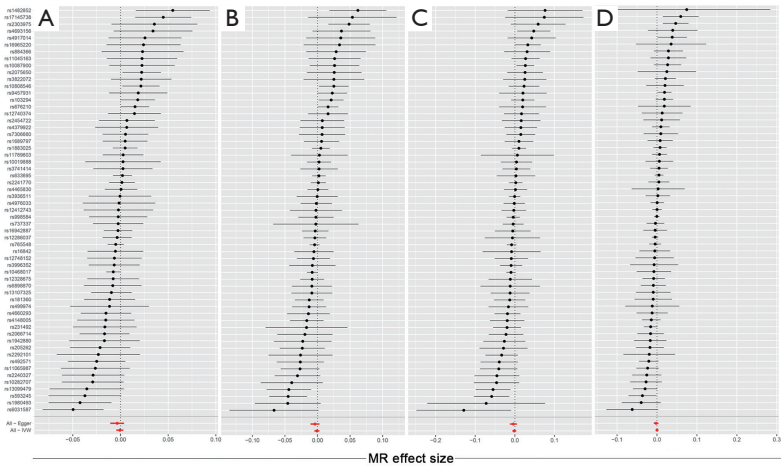

Figure S7 Forest plot of variant specific inverse variance estimates for causal association between HDLC and gallstone disease. (A) Variant specific inverse variance estimates for causal association between HDLC and gallstone disease from the Global Lipids Genetics Consortium and the UK Biobank cohort. (B) Variant specific inverse variance estimates for causal association between HDLC and gallstone disease from the UK Biobank cohort. (C) Variant specific inverse variance estimates for causal association between HDLC and gallstone disease from women-specify populations in the UK Biobank cohort. (D) Variant specific inverse variance estimates for causal association between HDLC and gallstone disease from men-specify populations in the UK Biobank cohort. HDLC, high-density lipoprotein cholesterol; IVW, inverse variance-weighting; MR, Mendelian randomization.

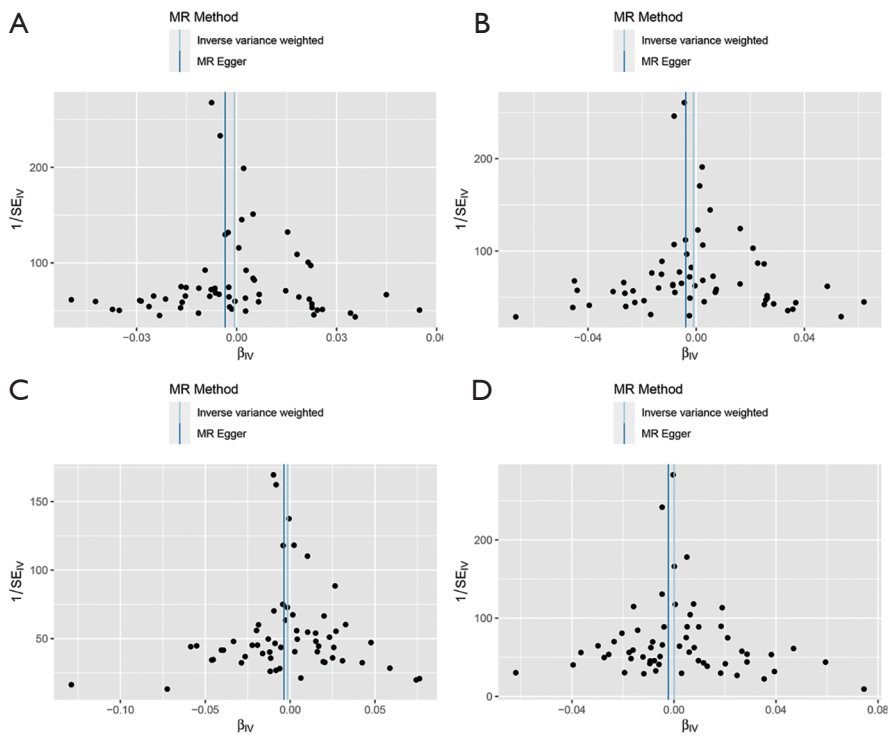

Figure S8 Funnel plot of causal association between HDLC and gallstone disease. (A) Funnel plot for causal association between HDLC and gallstone disease from the Global Lipids Genetics Consortium and the UK Biobank cohort. (B) Funnel plot for causal association between HDLC and gallstone disease from the UK Biobank cohort. (C) Funnel plot for causal association between HDLC and gallstone disease from women-specify populations in the UK Biobank cohort. (D) Funnel plot for causal association between HDLC and gallstone disease from men-specify populations in the UK Biobank cohort. HDLC, high-density lipoprotein cholesterol; MR, Mendelian randomization; SE, standard error.

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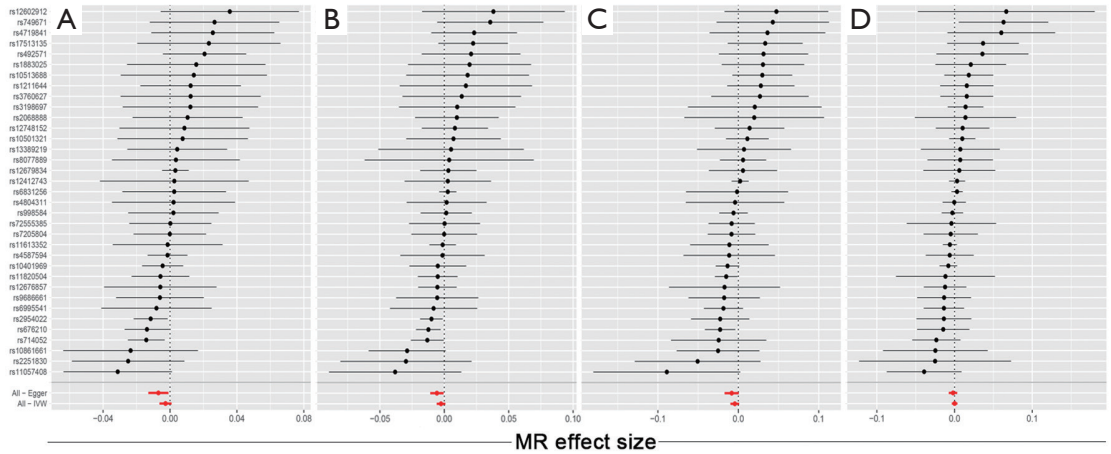

Figure S9 Forest plot of variant specific inverse variance estimates for causal association between triglycerides and gallstone disease. (A) Variant specific inverse variance estimates for causal association between triglycerides and gallstone disease from the Global Lipids Genetics Consortium and the UK Biobank cohort. (B) Variant specific inverse variance estimates for causal association between triglycerides and gallstone disease from the UK Biobank cohort. (C) Variant specific inverse variance estimates for causal association between triglycerides and gallstone disease from women-specify populations in the UK Biobank cohort. (D) Variant specific inverse variance estimates for causal association between triglycerides and gallstone disease from men-specify populations in the UK Biobank cohort. IVW, inverse variance-weighting; MR, Mendelian randomization.

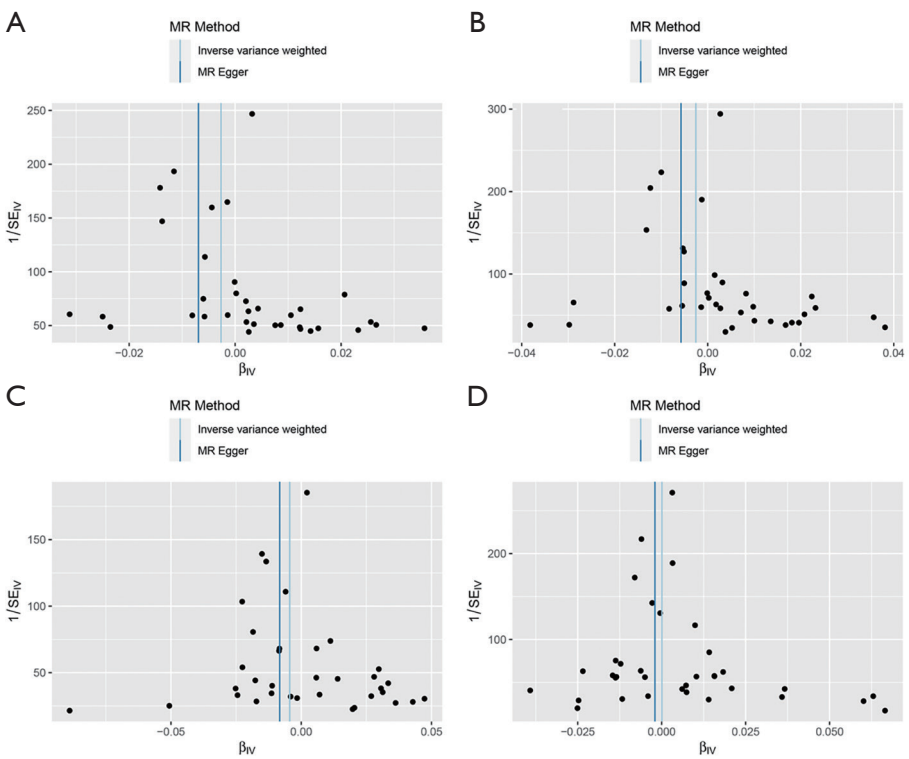

Figure S10 Funnel plot of causal association between triglycerides and gallstone disease. (A) Funnel plot for causal association between triglycerides and gallstone disease from the Global Lipids Genetics Consortium and the UK Biobank cohort. (B) Funnel plot for causal association between triglycerides and gallstone disease from the UK Biobank cohort. (C) Funnel plot for causal association between triglycerides and gallstone disease from women-specify populations in the UK Biobank cohort. (D) Funnel plot for causal association between triglycerides and gallstone disease from men-specify populations in the UK Biobank cohort. MR, Mendelian randomization; SE, standard error.

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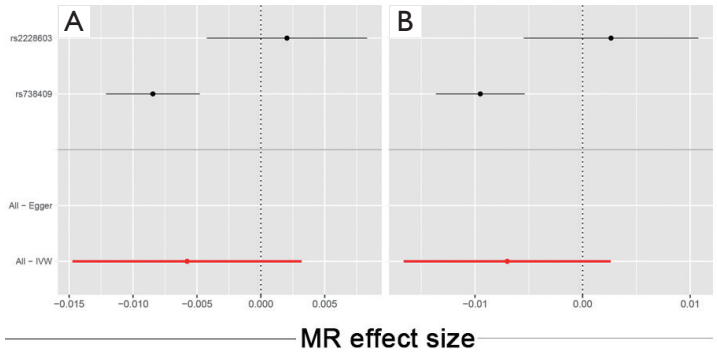

Figure S11 Forest plot of variant specific inverse variance estimates for causal association between liver fat content and gallstone disease. (A) Variant specific inverse variance estimates for causal association between hepatic steatosis and gallstone disease from the Genetics of Obesity-related Liver Disease and the UK Biobank cohort. (B) Variant specific inverse variance estimates for causal association between non-alcoholic fatty liver disease and gallstone disease from the Old Order Amish, Age, Gene/Environment Susceptibility-Reykjavik study and the UK Biobank cohort. IVW, inverse variance-weighting; MR, Mendelian randomization.

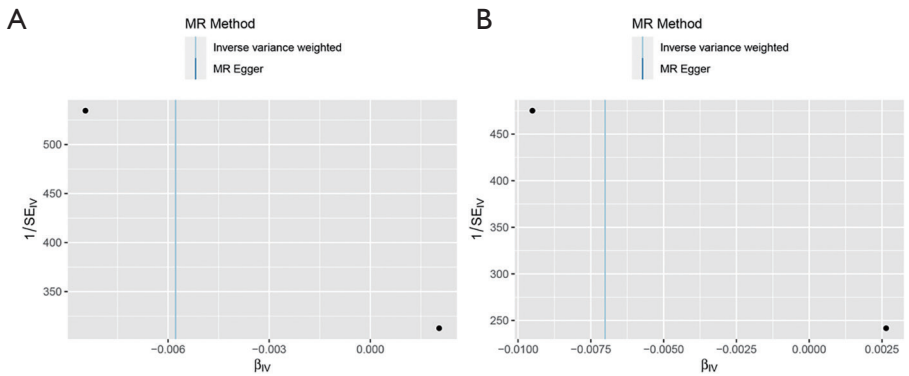

Figure S12 Funnel plot of causal association between liver fat content and gallstone disease. (A) Funnel plot for causal association between hepatic steatosis and gallstone disease from the Genetics of Obesity-related Liver Disease and the UK Biobank cohort. (B) Funnel plot for causal association between non-alcoholic fatty liver disease and gallstone disease from the Old Order Amish, Age, Gene/Environment Susceptibility-Reykjavik study and the UK Biobank cohort. MR, Mendelian randomization; SE, standard error.

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Table S5 Mendelian randomization estimations showing the effect of lipid profiles on GSD in combined sex

Exposure	Methods	Odds ratio[a]	95% CI		P value	Ph	Q-statistics
LDLC	IVW	0.994	0.991	0.997	4.15E–04	1.03E–02	63.5
	MR-Egger	0.994	0.990	0.998	7.03E–03	7.98E–03	63.4
	Weighted median	0.996	0.992	1.000	3.70E–02	–	–
	Simple median	0.996	0.991	1.001	1.02E–01	–	–
	MR-Egger intercept[b]	0.0001	–0.0002	0.0003	8.32E–01	–	–
HDLC	IVW	0.999	0.995	1.003	6.25E–01	2.91E–04	102.4
	MR-Egger	0.996	0.989	1.003	2.71E–01	3.26E–04	100.6
	Weighted median	0.996	0.992	1.001	1.21E–01	–	–
	Simple median	0.998	0.993	1.003	3.67E–01	–	–
	MR-Egger intercept[b]	0.0002	–0.0001	0.0004	3.16E–01	–	–
Triglycerides	IVW	0.997	0.994	1.001	1.30E–01	3.87E–01	35.7
	MR-Egger	0.994	0.989	0.999	3.52E–02	4.55E–01	33.2
	Weighted median	0.999	0.993	1.004	6.36E–01	–	–
	Simple median	1.003	0.996	1.009	4.29E–01	–	–
	MR-Egger intercept[b]	0.0001	–0.0001	0.0005	1.25E–01	–	–

[a], odds ratio per 1 SD increase; [b], regression coefficient (95% CI). CI, confidence interval; GSD, gallstone disease; IVW, inverse variance-weighting; LDLC, low-density lipoprotein cholesterol; HDLC, high-density lipoprotein cholesterol; MR, Mendelian randomization; Ph, P value for heterogeneity; SD, standard deviation.

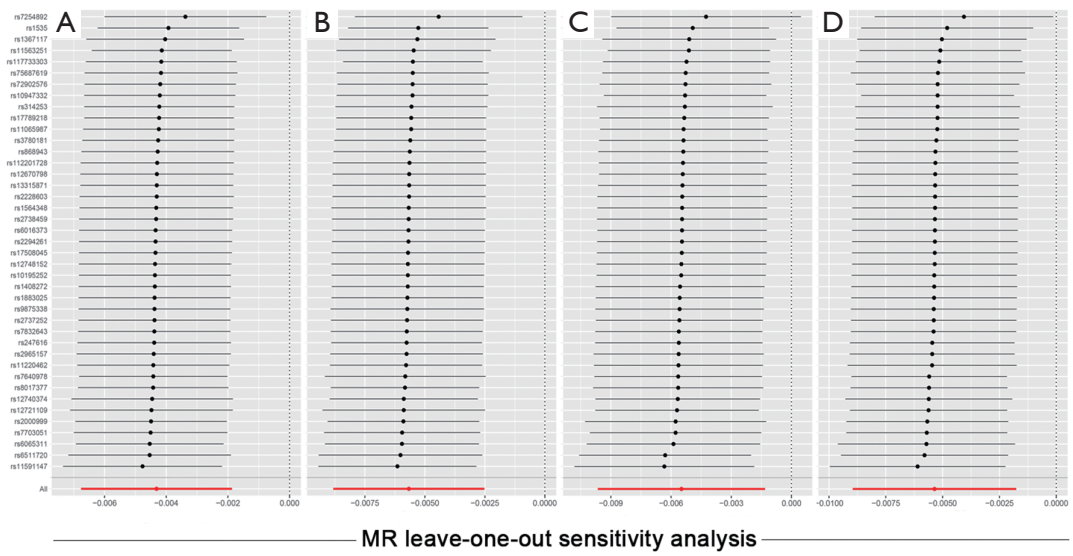

Figure S13 Leave-one-out plot to assess if a single variant is driving the association between LDLC and gallstone disease. (A) Leave-one-out plot to assess the two-sample MR causal estimation between LDLC and gallstone disease from the Global Lipids Genetics Consortium and the UK Biobank cohort. (B) Leave-one-out plot to assess the one-sample MR analysis causal estimation between LDLC and gallstone disease from the UK Biobank cohort. (C) Leave-one-out plot to assess the one-sample MR analysis causal estimation between LDLC and gallstone disease from women-specify populations in the UK Biobank cohort. (D) Leave-one-out plot to assess the one-sample MR analysis causal estimation between LDLC and gallstone disease from men-specify populations in the UK Biobank cohort. MR, Mendelian randomization; LDLC, low-density lipoprotein cholesterol.

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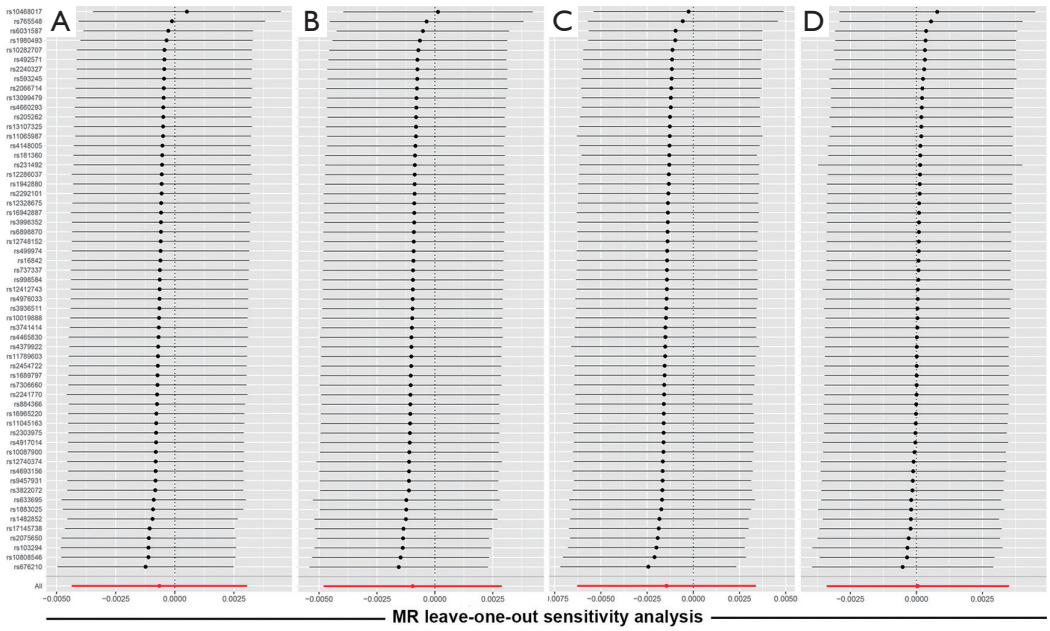

Figure S14 Leave-one-out plot to assess if a single variant is driving the association between HDLC and gallstone disease. (A) Leave-one-out plot to assess the two-sample MR causal estimation between HDLC and gallstone disease from the Global Lipids Genetics Consortium and the UK Biobank cohort. (B) Leave-one-out plot to assess the one-sample MR analysis causal estimation between HDLC and gallstone disease from the UK Biobank cohort. (C) Leave-one-out plot to assess the one-sample MR analysis causal estimation between HDLC and gallstone disease from women-specify populations in the UK Biobank cohort. (D) Leave-one-out plot to assess the one-sample MR analysis causal estimation between HDLC and gallstone disease from men-specify populations in the UK Biobank cohort. MR, Mendelian randomization; HDLC, high-density lipoprotein cholesterol.

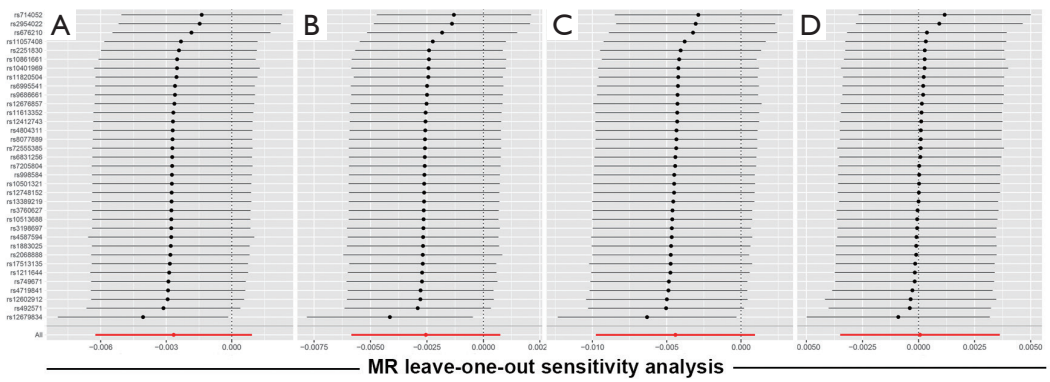

Figure S15 Leave-one-out plot to assess if a single variant is driving the association between triglycerides and gallstone disease. (A) Leave-one-out plot to assess the two-sample MR causal estimation between triglycerides and gallstone disease from the Global Lipids Genetics Consortium and the UK Biobank cohort. (B) Leave-one-out plot to assess the one-sample MR analysis causal estimation between triglycerides and gallstone disease from the UK Biobank cohort. (C) Leave-one-out plot to assess the one-sample MR analysis causal estimation between triglycerides and gallstone disease from women-specify populations in the UK Biobank cohort. (D) Leave-one-out plot to assess the one-sample MR analysis causal estimation between triglycerides and gallstone disease from men-specify populations in the UK Biobank cohort. MR, Mendelian randomization.

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Table S6 Multivariable mendelian randomization estimations showing the effect of plasma lipid profiles on GSD

Exposure	Odds ratio[a]	95% CI		P value
LDL-cholesterol				
Two-sample MR[b]	1.001	1.000	1.003	1.18E−01
Combined[c]	0.993	0.990	0.996	0.00E+00
Men[c]	0.995	0.991	0.998	1.00E−03
Women[c]	0.992	0.989	0.996	0.00E+00
HDL-cholesterol				
Two-sample MR[b]	0.998	0.995	1.001	2.18E−01
Combined[c]	0.998	0.996	1.001	1.30E−01
Men[c]	1.001	0.999	1.004	3.36E−01
Women[c]	0.996	0.993	0.999	1.30E−02
Triglycerides				
Two-sample MR[b]	1.005	1.001	1.008	1.20E−02
Combined[c]	0.999	0.996	1.002	6.28E−01
Men[c]	1.002	0.999	1.005	2.60E−01
Women[c]	0.998	0.993	1.002	2.88E−01

[a], odds ratio per 1 SD increase; [b], two-sample MR between GLGC and UK Biobank; [c], one-sample MR in UK Biobank. CI, confidence interval; GLGC, Global Lipids Genetics Consortium; GSD, gallstone disease; LDL, low-density lipoprotein; HDL, high-density lipoprotein; MR, Mendelian randomization; SD, standard deviation.

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