Peer Review File

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Reviewer 1:

An interesting read on the use of mendelian randomization to examine the causal effect of dyslipidemia and NAFLD on gallstone disease. No comments on the study design but some of my suggestions on the phrasing include:

Point 1. line 40: If the CI for LDLC does not cross 1.0, there is a negative association for LDLC and GSD and should be reported as such in the abstract and not NO causal relationship

Response: Thank you very much for your kind question. In this study, we obtained the results that the odds ratio of LDLC was 0.995 (95% CI: 0.994-0.998) that was very close to 1.0. Therefore, we deemed that it might not affect our final conclusion.

Point 2. line 54: Gallstone disease can be complicated by cholecystitis, cholangitis, pancreatitis and also gallbladder cancer.
(would be informative if the authors can also mention what the disease burden of GSD is, how many cholecystectomies are performed per annum / cost of treatment etc)

Response: Thanks for the kind suggestions. We searched the previous reviews about gallstone disease and revised the manuscript suggested above.

Point 3. line 69: It is not accurate that lipid lowering drugs are used in the treatment of patients with asymptomatic gallstones. Is there any evidence to back up this statement?

Response: Thanks for the kind suggestion. We added the evidence derived from Michael et al. to show the widely administration of lipid lowering drugs for asymptomatic gallstone patients.

Reviewer 2:

Point 1. Line 37: Please explain difference of case number between case and control, does it influence the result of statistic power?

Response: Thank you very much for your kind question. In this study, we recruited the data from 10,520 cases and 361,194 controls from UK Biobank that is the largest database of available prospective cohort studies. Similarly, Milad Nazarzadeh et al. have previously utilized this database to analyze the risk of plasma lipids in aortic valve stenosis, and they compared from 1961 cases and 432,173 controls. (*Eur Heart J. 2020 Oct 21;41(40):3913-3920.*) Additionally, Liu et al. also analyzed 1,122 cases and 399,900 controls in this database to show the causal relationships between nonalcoholic fatty liver disease (NAFLD), type 2 diabetes and obesity. (*J Hepatol. 2020 Aug;73(2):263-276*) Therefore, although it certainly exists the mismatching problem between these two groups in this study, we resulted that hyperlipidemia or nonalcoholic fatty liver disease (NAFLD) is not causally associated with gallstone disease. And a prospective study with a larger sample size is needed for future research.

Point 2. Line 249: The instrument variables only explain approximately 3%to 8% of the variance of exposure, please explain why such data can be used.

Response: Thank you very much for your kind question. Available evidence demonstrated that the risk factor for growing gallstones is, indeed, not only genetic predisposition, which also involve the factors derived from metabolic and anatomic abnormalities, parasitic infection, and environmental factors, and so on. (*Nat Rev Dis Primers. 2016 Apr 28;2:16024*) And Mendelian randomization (MR) is well-known as a technique for causal inference that utilize genetic variants as instrumental variables to analyze the causal weight of the risk factor in an outcome using observational data so that infer the unconfounded correlation between an exposure and outcome. (*BMC Med. 2020 Nov 10;18(1):312*) Based on gallstones is a disease leading by the interaction of multiple pathogenic factors, we herein obtained the observed phenotypic variances of LDL, HDL and TG were respectively 6.90%, 3.67% and 4.27%. And the two SNPs explained 3.2% of the variance in hepatic steatosis was also observed. The instrument variables explain approximately 3%-8% of the variance of exposure, which was consistent with the previous study of Mendelian randomization study of the association between adiposity and cardiovascular outcomes reported by Kim et al. (the proportion of variance in his study was 1.6-1.82%). (*Eur Heart J. 2021 Sep 7;42(34):3388-3403*)

Moreover, we additionally calculated the F-statistic values for these four variables, which respectively were 150.3, 59.9, 116.7, and 118.56, greater than 10, indicating that the selected variables possess sufficiently strong enough effect size to serve as valid instruments. And a larger sample size of GWAS data for further MR analysis to detect the relationship between lipid profiles and gallstones disease is required.

Point 3. Line 256: Conclusion need to state limitation of this study.

Response: Thanks for the kind suggestions. According to the reviewer's suggestion, we modified the conclusion in the revised manuscript. We deemed that there are three limitations in this study, as follow: 1) although our findings are consistent with hyperlipidemia and NAFLD on GSD in two-sample MR and one-sample MR, the instrument variables only explain approximately 3% to 8% of the variance of exposure and this study may have been underpowered to detect medium to small effects. 2) 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. 3) by using the GLGC study and UK Biobank cohort, the majority of participants in our research were of European ancestry and we were unable to investigate the relationship between lipid profiles and GSD in Asian and African populations.

Point 4. Line 32 and 35: NAFLD and SNP need full name while first appeared.

Response: Thanks for the kind suggestions. I'm sorry for our oversight while preparing the previous manuscript. As suggested, we added the fully names of NAFLD and SNP in the first appeared in the revised manuscript.