Reviewer's report

Title: Association between bilirubin and cardiovascular disease risk factors: Using Mendelian randomization to assess causal inference

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Reviewer: So-Youn Shin

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The authors used Mendelian randomization method to test a hypothesis that genotype variants affecting some CVD risk factors would be mediated by bilirubin levels. For this analysis, UGT1A1*28 was used as an instrumental variable for bilirubin, because 1. the gene function is known to be associated with bilirubin and 2. the allelic variation at this locus explains 45% of the variance of bilirubin in this population.

The aim of the study for causal inference is clearly stated (i.e. the association would be due to confounding or reverse causality, and without distinguishing causality from these, the use of bilirubin would be limited to identify underlying pathway in CVD), and the materials and methods, such as phenotype measurement, are well described.

Discretionary Revisions

1. A dominant inheritance model was assumed in the analyses but the reason was not stated. Is it for the power of the study or for other reasons? A dominant model assumption makes sense according to bilirubin in table 2 (and brachial artery width in table 3), but an additive model with a dominance genetic value can be also considered. Especially since a slight difference of bilirubin level (and brachial artery width) is observed between 6/6 and 6/7 genotypes – the significance for this difference should be tested however.

2. The adjustment for relatedness using variance components model is a reasonable choice, but the degree of relatedness is missing.

3. The limitation of the third assumption in Mendelian randomization is mentioned briefly in Materials and Methods, but this would be worth to be readdressed in Conclusion.

Minor Essential Revisions

None

Major Compulsory Revision

1. P-value

A total of 16 risk factors were tested. However, the selection criteria can’t be
found in the manuscript. Are these all the traits tested and available? Or the ones of interest? If so, is it based on literature?

As multiple traits were tested for association with bilirubin, false positive rate might increase unless the significance level was adjusted. For example, 0.05/16=0.0031 can be suggested.

If this threshold is applied, MR approach would give us different results. In my opinion, the association of UGT1A1*28 genotypes (through bilirubin) and CPT is not significant in this study, and thus should be taken out of the conclusion.

On the other hand, the traditional association test result would be the same (i.e. BMI, LDL-C and total cholesterol are still associated with bilirubin).

Another reason I think CPT might be a false positive (i.e. might not be in a causal relation with bilirubin), is because the mean CPT doesn’t increase as the number of 7 genotype increases. In this respect, the brachial artery width is likely to be a true positive.

2. Validation

No validation study for the model (such as cross validation) or for data (such as replication) was conducted. The impact of this study then would be very limited until another independent study replicates the result. The authors may have reasons for lack of validation in the analysis.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests.