Reviewer's report

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

Version: 2 Date: 8 November 2011

Reviewer: Carlo Berzuini

Reviewer's report:

The paper offers an interesting application of Mendelian Randomization to the assessment of a possible causal influence of elevated serum bilirubin (ESB) on risk factors for Cardiovascular Disease (CVD). Use of a sample of healthy individuals offers some protection against possible bias due to reverse causality, and Mendelian Randomization offers some protection against possible biases due to unobserved confounding.

Overall, the paper is well written, the methodology is applied in a correct way to an interesting substantive problem. The authors discuss the assumptions carefully, in the light of relevant background knowledge. I believe the paper deserves publication on BMC, provided the authors clarify (in a response letter and - if appropriate - in their manuscript) the issues raised below. The following points should not be interpreted to indicate inadequacy of the reviewed work, but simply suggestions of possible improvement. I would be seriously upset if the points below were used to support a case for paper rejection.

1) you say you have analyzed a sample of relatively healthy Old Order Amish individuals. Which means that the analysis is, in a sense, conditional on absence of CVD. Do the authors feel that this conditioning (on a causal descendant of the outcome variable) may introduce possible biases? Could a "rare disease assumption" help here?

2) the causal diagram shown in Figure 1 is very helpful. The usefulness of these diagrams in epidemiological studies cannot be overstated. The authors have decided not to include in the diagram the variables Brachial Artery width (BAW) and cold pressor test (CPT), despite the fact that these two variables play a role in the discussion. The reason I raise this issue is that the paper seems to suggest that UGT1A1*28 influences the risk factors for CVD though BAW and CPT. Am I correct? If so, are we not in front of an alternative causal path from UGT1A1*28 to the CVD risk factors, different from the one that goes through ESB? If my interpretation is correct, in order to comply with the assumptions for validity of Mendelian Randomization, one could consider some form of adjustment for BAW and CPT, although, unfortunately, such an adjustment might give way to a collider bias. In the case where my interpretation is correct, I do not regard the study to be flawed; I simply ask the authors to spend one or two sentences on this issue, clarifying the extra assumptions that the detected issue...
calls for. All causal studies suffer from difficulties of this sort.

3) phrases like "brachial artery width", "baseline brachial artery diameter", "brachial artery diameter" appear in different parts of the text. I am a bit confused. Do they mean the same thing? If so, why not using a single acronym throughout? If they are not the same thing, shouldn't the reader have some insight of the causal relationships between the entities, in relation to the causal diagram? What are the relationships between these entity (or entities) and the outcome variable? This issue is evidently related to the preceding one.

4) Does CPT stand for "cold pressure reactivity"?

MINOR POINTS AND TYPOS

Page 3, line 1: I am not a UK native, so I might be wrong, but I prefer the expression "reduced risk of cardiovascular disease" to "reduced cardiovascular disease". Apologies if I am wrong.

Page 10, second line of the RESULTS section, should be "known"