Reviewer’s report

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

**Version:** 2  **Date:** 7 October 2011

**Reviewer:** Frank Dudbridge

**Reviewer’s report:**

The authors report a Mendelian randomization study of the causal effect of bilirubin on a number of cardiovascular risk factors, in an Amish population. Significant observational associations between bilirubin and BMI, LDL and total cholesterol were not confirmed by the MR analysis. However there were significant MR associations for brachial artery width and blood pressure, despite no observational association.

The paper is well written and uses standard methodologies. A potential complication from using related subjects was dealt with appropriately with a variance components model.

**Major compulsory revisions:**

1. P11 "genotype accounts for 45% of the variation" - clearly a strong instrument, would be good to also report the corresponding F statistic

2. P13 "confounding is severe enough to reverse the direction" - firstly the statement is rather strong given that the MR estimates were not significantly different from zero (so we don’t reject the hypothesis that the effects are in the same direction". Secondly, could power estimates be given based on the estimated gene-phenotype association, phenotype-outcome associations (assuming no confounding), gene frequency and sample size? This would reassure us that the non-significant MR tests are due to lack of causal effect, rather than lack of power.

3. Much is made of the brachial artery MR association, yet there is no observational association. There should be some discussion of this anomaly. Also, given the lack of prior evidence for causality on brachial artery (and also blood pressure), there ought to be a multiple test correction applied.

4. Parts of table 3 look wrong, eg MR p-value of 0.65 when all three genotypes have the same mean and the point estimate is zero, and CIs that touch 0 for waist circ and pulse wave, but do not have significant p-values - please double-check all the numbers.

**Minor Essential Revisions**

5. P14 L-4 and elsewhere, "increase reactivity" should be "increased reactivity"
this grammatical occurs too often to be a simple typo (and also for "decreased")

6. P13 L-8 "This study supports the hypothesis that increased bilirubin levels cause a decreased risk of CVD" - this is simply not the case, as there are no results on CVD risk and most of the results for causality on CVD markers are negative.

Discretionary Revisions

7. P4 L3 "associated with increased risk ... bilirubin acts to protect against CVD" reads confusingly, suggest rewording eg "high levels of bilirubin are associated with decreased risk ...

Level of interest: An article of importance in its field

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