Reviewer's report

Title: Maternal and offspring fasting glucose and type 2 diabetes-associated genetic variants and cognitive function at age 8: a Mendelian randomization study in the Avon Longitudinal Study of Parents and Children

Version: 1 Date: 24 April 2012

Reviewer: Michelle Luciano

Reviewer's report:

General Comments
This study investigates previously reported links between T2D and fasting glucose levels with cognitive ability by assessing the impact of genetic variants influencing the former measures on the latter. I can't see how this is a Mendelian Randomization (MR) study though, when the exposure variables (glucose concentration, HbA1c fraction) don't predict the outcome variable (IQ). No MR test can be done. The study seems to be the preliminary analyses one performs before directly testing a MR model. If one wants to emphasize the MR approach, then the study needs to be clear on what it expects to observe - i.e., the specific relationships between the instrumental variables, the confounders and the outcome variable – in order to formulate a MR model; and then discussion of whether the assumptions hold and what the findings imply. The study has a lot of merit though, with the novel use of T2D/fasting glucose genetic risk to predict IQ (one may have even hypothesised pleiotropy here). The lack of an association between the exposure variables and childhood cognitive ability suggests that the cognitive decline often observed in T2D/related traits is age dependent, perhaps building up over time, this could be discussed.

MAJOR COMPULSORY REVISIONS

Methods:
1. Were the adjustments in the analysis using maternal diabetes/glycosuria all significant covariates, why were these selected exactly, what of maternal education and other confounders previously mentioned?
2. The next sentence is confusing. Which genotype of the child are you controlling for? Why are you controlling for genotype rather than childhood glucose?

Results:
3. Was the difference between breastfeeding in diabetic and non-diabetic mothers significant? Could it be explained by maternal education or are there other reasons why diabetic mothers prefer not to breastfeed?
4. While I can understand why maternal risk scores might be associated with gestational age, what is the rationale for examining the association with offspring’s sex (what does this mean exactly)?
5. In the next sentence are you referring to offspring’s genetic risk scores for both glucose and T2D? Presumably, some of the covariates (e.g., drinking and smoking) are not independent so what correction did you use for the Bonferroni adjustment?

6. After reading this section on confounders I was left not knowing which covariate associations you considered true effects. For the genotype/risk score results, the concluding sentence of this paragraph does not mesh with the opening sentence. The lack of association between genotype and confounders is an important assumption of MR, so this section needs to be clearer.

7. You mentioned maternal education in the Methods, but no results for this were included. Wouldn’t this be the largest confounder of them all?

8. Does a genetic risk score based only on the significant individual SNPs in your study explain more variance than the FGGRS and T2DRS?

9. How exactly did you arrive at the 4.5 IQ point lowering for every increase of 1mmol/l in fasting glucose, and how is your estimate consistent when it’s not significantly different from 0?

10. If maternal education is associated with diabetes/glycosuria status of mothers then it should be included as a covariate in the analysis of mother’s diabetes and child’s IQ, as it will also correlate with offspring’s IQ.

Discussion:

11. Did you really lack statistical power to confirm an association between fasting glucose and IQ, I would have thought not, what was the power for the effect size you were expecting? Similarly, if you were able to confirm a small effect size for FGGRS and fasting glucose doesn’t this suggest your sample was sufficiently powered? A larger sample will obviously detect smaller effects, though.

12. The derivation of the MR expectation is not clear to me.

13. You state that the negative association between diabetes and cognition could be the result of confounding, what variables were you thinking of here?

MINOR ESSENTIAL REVISIONS

Background:

1. The first sentence needs citations.

2. How much variance do the GWAS candidate genes explain in glucose levels and T2D?

3. The use of ‘and/or’ in the second sentence of the final paragraph is confusing.

4. Why were only 16 and 22 variants used to form the genetic risk score?

5. The last sentence should state that the causal relationship investigated is with ‘childhood’ cognitive ability.

Methods:

6. Why were only 846 children with fasting glucose included in the study?

Why did only 1793 children from 7314 have HbA1c and why were only 1454
included in the study?
7. I don’t think the ‘++’ coding in the medical records needs to be mentioned, the measurement threshold will suffice.
8. Did 3 SNPs fail genotyping in mothers, why this difference?
9. Were the population stratification PCs derived only from the genotyped SNPs because the reference given refers to GWAS?
10. The Barker et al ref. shouldn’t have the year.
11. In calculating genetic risk scores what happened if data were missing for particular SNPs?
12. Wouldn’t the genetic risk score for T2D be calculated from SNPs described in Hivert et al AND the GWAS catalogue rather than OR? How many came from each?
13. What was the LD for variants that were excluded from the scores?
14. The T2DGRS score is missing rs before the 2nd last marker in the formula.
15. 16 + 22 markers gives 38 markers, but you state that 3 markers were excluded because they weren’t typed in mothers, which gives 35 available markers in total. Does this mean there is an overlap in SNPs used in the FGGRS and T2DGRS scores?

Results:
16. Be clear whether the SNPs out of HWE were excluded.
17. What were the categories of breastfeeding duration considered? What is the reported mean difference of .07 mmol/l a difference between?
18. For the maternal diabetes association with gestational age, what is -0.28 supposed to mean in terms of direction, that non-diabetic mothers gave birth to lower gestational age offspring? Ditto for birth weight.

Tables
19. Italicize gene names
20. Table 1: Report the range of N typed for individual SNPs.
21. Table 2: Why do the Ns differ for analyses adjusted and not adjusted for population stratification?
22. Figure: What does the average in parentheses mean in the second last box, also a typo ‘withouth’
23. Additional tables: It would help to bold the nominally significant associations

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

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a
statistician.

Declaration of competing interests:

I declare that I have no competing interests.