Reviewer's report

Title: Type 2 diabetes gene TCF7L2 polymorphism is not associated with fetal and postnatal growth in two birth cohort studies.

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Reviewer: Rachel Freathy

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Mook-Kanamori and colleagues have examined the evidence for association between the common rs7903146 variant in TCF7L2 and measures of fetal and infant growth using subjects from a prospective, population-based study (N=3419) and a cohort of SGA subjects (N=566). They compared allele frequency between the two cohorts and found no difference (P=0.47). No association was found between TCF7L2 genotype and any fetal or postnatal growth measure in either study, or with longitudinal growth rates from 0 to 2 years (P>=0.04). The authors conclude that "TCF7L2 would not appear to be involved in the previously demonstrated associations of low birth weight with T2D".

Strengths of this paper include detailed fetal and infant growth phenotypes and reasonable sample power. The authors have tested a clear hypothesis using appropriate methods, and the manuscript is clear and well written.

I have the following points of concern:

Minor essential revisions:

1. Introduction, paragraph 2: "The T-allele of rs7903146, which has an estimated [minor] allele frequency in Caucasians of about 25%..." This is a little vague, and should be clarified by, for example, giving the MAF range from cited publications or quoting the HapMap CEU minor allele frequency.

2. Introduction, final sentence: the SGA cohort is referred to as "replication" study. I do not think this is an accurate description because of the different nature of ascertainment and, crucially, the sample size. It would be more appropriate to speak of using two independent studies to investigate the same question, rather than describing them as primary and replication studies.

3. Minor allele frequency was compared between the two studies. This analysis seems similar to a case-control study, comparing individuals who are small for gestational age with those who are appropriate for gestational age. However, the authors did not remove individuals who were small for gestational age from the "control" (Generation R) study. I realise that the small number of these is unlikely to make much difference to the results, but the rationale for this analysis, and the decision not to exclude SGA subjects from the Generation R study for this analysis should be explained more clearly.
4. It is not clear why the authors tested dominant and recessive models in addition to the additive model. I am not aware of any study which reports association between TCF7L2 and type 2 diabetes as deviating significantly from an additive model. Testing these additional genetic models without clear rationale for doing so increases the number of tests carried out, without adding value to the study.

5. The power calculation in the methods is helpful to the reader. However, there were fewer individuals available for the postnatal analyses, so presumably the effect size detectable was not as small. It would be more helpful to give a little more detail than "able to detect differences in growth characteristics of about 0.05 SDS", perhaps giving the effect size detectable in (i) the fetal and (ii) the postnatal growth analyses. Additionally, in terms of study design, a P value threshold of 0.05 is too lenient, considering the number of tests that have been carried out.

6. In Table 3, it would be helpful to include the numbers of subjects by genotype, as has been done in tables 4 and 5.

7. Was gestational age transformed for linear regression analysis? How was the distribution of this phenotype treated.

8. The authors mention in the results section that one P value was < 0.05 (for weight at 2 years in the SGA cohort). I am curious as to why this result is picked out. Multiple testing considerations mean that this result could easily have occurred by chance. I do not think it is worth mentioning here.

9. While I agree with the authors' conclusion (especially in the light of previous data) that fetal TCF7L2 genotype is not associated with fetal or infant growth (and therefore unlikely to influence metabolic phenotypes until after early childhood), I think it is important for the authors to qualify this with reference to the limits of detection in the current study. The confidence limits around their effect size estimates cannot rule out smaller effects of this variant on fetal growth. This is a minor point, but a statement in the discussion, acknowledging the power and detection limits of the study would be helpful.

10. It should be mentioned that the results are not adjusted for maternal genotype. As the authors point out, associations between the maternal TCF7L2 risk allele and increased offspring birth weight have previously been documented. Fetal and maternal genotypes are 50% correlated. This raises the possibility that, in utero, where the risk allele is present in both mother and fetus, any small effects of fetal genotype reducing fetal growth could be masked by opposing effects of maternal genotype. Again, in the light of previous data, this is unlikely, but the discussion should acknowledge that this possibility cannot be ruled out because maternal genotype was not available in the current study.

11. Discussion, page 7, paragraph 1: "Several studies investigated the effect of common genetic variants related to insulin action on early growth and found no or inconsistent associations [15,20,21], possibly because they investigated gene
polymorphisms that appear to be less strongly associated with T2D than TCF7L2 rs7903146." There are a few important points to make about this statement:

(a) I agree that power would be lower for variants that are less strongly associated with T2D (or more specifically, predispose to T2D with smaller effects) than TCF7L2, but it is important to make the distinction between the PPARG and KCNJ11 variants tested in reference 20 (which are confirmed diabetes susceptibility genes, verified genome-wide and widely replicated) and the IGF1 and INS-VNTR variants, which do not predispose to type 2 diabetes.

(b) KCNJ11 is generally thought not to associate with insulin action, but insulin secretion.

(c) A recently published study in Diabetes documents strong associations between the fetal CDKAL1 and HHEX T2D variants and birth weight (Freathy et al, 2009).

12. Figure 1 was unreadable from the pdf file, so I was unable to check it. This is a useful thing to include in the paper though.

**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.