Author's response to reviews

Title: A study of association between common variation in the growth hormone-chorionic somatomammotropin hormone gene cluster and adult fasting insulin in a UK Caucasian population

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Author's response to reviews: see over
Dear Editorial Team,

**MS: 5658000311045255: Failure to replicate an association between common variation in the growth hormone-chorionic somatomammotropin hormone gene cluster and adult fasting insulin in a UK Caucasian population**

Thank you for your email of 08.09.06, which enclosed the Reviewers’ comments. Please find attached the revised manuscript. We have highlighted changes made in yellow. Please note in particular that, in response to Reviewer 2’s comments, we have changed the title to “A study of association between common variation in the growth hormone-chorionic somatomammotropin hormone gene cluster and adult fasting insulin in a UK Caucasian population”. We have made no changes to Figure 1, submitted on 24.05.06.

Our responses to the points made by the Reviewers are included below.

We look forward to hearing from you.

Yours faithfully,

Rachel Freathy.
Reviewer 1

We thank Reviewer 1 for the helpful comments. Our responses to the points made are below and we have highlighted changes made at the appropriate points on the manuscript.

Major Compulsory Revisions

1. The failure to replicate genetic associations is a major current issue and have lead to calls for increased sample size and power not only for the original study, but also the replication studies. While the current study is clearly larger than that of Day et al., it is still relatively small. What are the 95% confidence limits for weight at 1 by genotype, in boys and girls? These are mentioned in the discussion but not shown. Girls with TT appear to have a 500-600 g reduction in weight at 1 consistent with the original study.

We agree that the sample size is still relatively small for a genetic association study of quantitative trait data. We acknowledge that further large-scale studies of the order of tens of thousands of samples are likely to be ultimately required to validate or refute such associations. We have added a sentence to acknowledge this in paragraph 2 of the Discussion. Whilst we had reduced power to detect differences in 1-year weight relative to the original study, our study was adequately powered to detect the differences observed by Day et al. (2004) in fasting insulin concentrations and our 95% confidence limits easily exclude the effect sizes initially observed.

We are sorry for the lack of clarity in displaying the 95% confidence limits for weight at 1 by genotype in boys and girls. These are now shown in Table 1 and we have added to the title of this table to make this clearer. We have also added the 95% confidence limits for the difference in 1-year weight between the TT and D2D2 homozygote males, referred to in paragraph 3 of the Discussion. We agree that girls in our study with the TT genotype had 500-600g reduction in weight. Indeed, the P-values of t-tests of the differences were P=0.023 (D1D1-TT; difference=546g (95%CI 77-1015) and P=0.075 (D2D2-TT; difference 601g (95% CI -64-1266)). Whilst these were consistent with the results seen for D2D2 vs TT males in the original study, there was no association observed in females in the same study (D1/T P=0.43; D2/T P=0.83) and the differences observed between the D and T homozygote groups were under 130g. We are reluctant to speculate further on the importance of this finding owing to the multiplicity of statistical
tests that have been carried out and the small sample size. We have added a comment about the results in females but wish to emphasise the caution with which these results are interpreted using the final sentence of paragraph 3 of the Discussion: “…further well-powered studies will be needed to confirm the role of this variant in fetal and infant growth.”

2. For purposes of replication it is important to use the same definition of the affected phenotype as the original study. The original study showed several significant associations, suggesting a likely role of the variant on Hyperinsulinaemia-related metabolic syndrome traits: including blood pressure, triglycerides and stimulated insulin and glucose levels. The current study should make clear that they can only test one aspect of this trait, i.e. fasting insulin.

We agree that a replication study should test the same phenotype as the original study. We also acknowledge that the original study showed associations in males (P<0.05, not corrected for multiple testing) between genotype and blood pressure, 30-min insulin and 30-min glucose, and suggestive associations between genotype and triglycerides (P=0.06-0.08). OGTT and blood pressure data were not available for our subjects and we have added this statement to the Results section, paragraph 3. We do, however, have triglyceride measures: there was no association between genotype and triglyceride levels in men (unadjusted or adjusted for age and BMI; all P > 0.4). We have added this to the Results section, paragraph 3. We acknowledge that we could only test a limited part of the insulin resistance phenotype and have added to the end of the Introduction to make it clear how we were measuring insulin resistance.

3. The difference in age between the populations (59 to 72 years in Day et al. vs. 27 to 36 in the current study) is an obvious source of heterogeneity as age has a marked effect on these metabolic outcomes. This should be highlighted in the abstract and conclusions.

We agree with the Reviewer that age is a potential source of heterogeneity. We have added to the Abstract (paragraph 3) and the Conclusion (paragraph 1) to highlight this.

4. A further association in the original study was with shorter adult height. What was the association in the current study?

For males in this study (results given as mean height (95% CI)): 
<table>
<thead>
<tr>
<th>D1D1</th>
<th>D1T</th>
<th>TT</th>
<th>N</th>
<th>ANOVA P</th>
<th>Regression P</th>
</tr>
</thead>
<tbody>
<tr>
<td>177.7 (176.8-178.7)</td>
<td>178.0 (177.2-178.9)</td>
<td>178.8 (177.0-180.6)</td>
<td>478</td>
<td>0.57</td>
<td>0.31</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D2D2</th>
<th>D2T</th>
<th>TT</th>
<th>N</th>
<th>ANOVA P</th>
<th>Regression P</th>
</tr>
</thead>
<tbody>
<tr>
<td>178.4 (176.3-180.4)</td>
<td>178.4 (177.1-179.7)</td>
<td>178.8 (177.0-180.7)</td>
<td>201</td>
<td>0.921</td>
<td>0.73</td>
</tr>
</tbody>
</table>

We have stated, in paragraph 3 of the Results section, that we did not observe an association with adult height.

**Minor Essential Revisions**

5. Results: para 3 should clarify that these results are not actually shown.

We have added this statement to the Results section, paragraph 3.
Reviewer 2

We thank Reviewer 2 for his supportive and constructive comments and have responded to the points raised below. We have highlighted changes made at the appropriate points in the text.

Minor Essential Revisions

My main points are that the insulin aspect of the study is NOT a replication, as the title would suggest, 30 year olds and 65 year olds are quite different and age dependence/divergence of genotypes is well recognised and traits of metabolic syndrome certainly increase very markedly with age. In single gene disorders, there are numerous examples, and even in insulin resistance this is well recognised (see for example PARL(PSARL). I propose that the title be modified to 'a study of..' rather than its present incorrectly assertive form. A clearer recognition of this point in the discussion with appropriate references should also be made.

We acknowledge that the difference in age between our adult male subjects and those in the previous study is a potential reason for the differences observed in the results. We have changed the title as suggested and have added to paragraph 2 of the Discussion so that it now reads as follows:

“Another factor which may account for the differing result is that adult males in our study were younger (median age 33 years) than those in the previous study (age range 59-72 years). It is possible that the relationship between genotype and fasting insulin is modified by age. Some studies have reported gene-age interactions after results across all ages showed a weak association, for example the recent study of the relationship between the Leu262Val variant in the PSARL gene and plasma insulin in human subjects [1]. As with simple gene-phenotype associations, these interactions require replication. To investigate this possibility further, it will be necessary to carry out large-scale studies of CSH1.01 and fasting insulin in individuals spanning a wide range of ages.”

Technically, the study of early life weight is not a negative result but an uninformative result through insufficient statistical power and one could then argue destined for a JURB instead of JNRB [where U=uninformative]. The authors do acknowledge this and I do acknowledge that there would have been sufficient power to detect a somewhat larger effect. It would be harsh though to enforce this! On the other hand, there are differences in males which
in a larger study could be around 2% magnitude, albeit the opposite way from our original study. In fact (unpublished) we have studied this marker in another study, and a tag for it in a third, and in every study have seen significant differences for boys (but not girls), but not in the same direction. Since the cohorts are from different locations and eras, genotypes of this genomic region may interact with early weight and other traits in a complex fashion and we are currently engaged in a larger scale study. I think the authors should add a couple of lines of discussion about the point that although not reaching any significance, the mean values for each genotype are not equal and furthermore there is a steady trend of values across male T/D1 groups.

We agree that our lack of statistical power for the 1-year weight study precludes us from drawing any conclusions about the nature of the association, but wish to re-emphasize that our study was very well powered to replicate the effect size on fasting insulin seen in the original study. We look forward to hearing the results of the Reviewer’s larger-scale study on infant weight. We acknowledge that there is a trend of values across the male T/D1 groups and have added to the Discussion (paragraph 3) as suggested.