Author’s response to reviews

Title: The longitudinal association of common susceptibility variants for type 2 diabetes and obesity with fasting glucose level and BMI

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Author’s response to reviews: see over
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Dear Dr Norton

Thank you for considering our manuscript entitled “The longitudinal association of common susceptibility variants for type 2 diabetes and obesity with fasting glucose level and BMI” (MS: 996972043656483) for publication in BMC Medical Genetics. I apologise again for the delay and inconvenience resulting from the error in the email address that I supplied, and thank you kindly for extending our deadline for revisions.

We have addressed the comments and suggestions of the editor and each of the reviewers below, and have made changes to the manuscript using tracked changes.

I look forward to hearing from you.

Yours sincerely

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Editor’s comment:

You wrote in your cover letter that you would need to seek permission from Diabetologia to replicate Tables 1 and 2. Please can you let us know if this permission has been received?
Permission has now been received by Springer to reproduce Tables 1 and 2.

Reviewer 1:

Discretionary Revisions

1. Since the authors could not find any longitudinal association of common susceptibility variants for T2D and obesity with fasting glucose level and BMI the authors may possibly reflect this in the manuscript title.
We feel that statement of the negative finding is best given in the context of the power of the study, so have left the title unchanged.

Minor Essential Revision

2. In the “Statistic power” sub-section the authors stated which difference in beta coefficient could be detected. Since the data are natural log-transformed it would be helpful for the reader if approximate effect size was provided in non-log units as well (mmol/l fasting glucose, kg/m2 BMI).
We agree that this would be a helpful conversion to report. However, as the beta coefficients and power values are very small (<0.01), back transformation does not give good estimates in natural units. The exponential (inverse log) of increasingly small positive values approaches 1, rather than 0 (the exponential of 0 = 1). Thus the minimum value possible for the back transformed power/beta coefficients is 1 (kg/m2 for BMI, mmol/L for fasting glucose), which is misleadingly high. For this reason, we feel that it’s best to report the data in log-transformed units.

3. Please report the SNP call rates e.g. as an extra column in ESM Table 1 and provide the genotyping validation method e.g. in the “Population characteristics” sub-section.
These revisions have been made as suggested.

4. Please, also report if the risk allele frequencies are comparable to other populations or if you observe remarkable differences.
The risk allele frequencies of all eight SNPs in the BHS cohort studied are comparable to those in European populations, as reported in the dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/). This observation has been added to paragraph 5 of the Discussion.

5. Please correct in ESM Table 1 in the “Unrelated, 18-80 year old population” part the SNP numbers for KCNJ11 and PPARG since it seems that the authors accidentally state two SNPs from GCK and APOA5.
These labelling errors have been corrected. The statistics reported in the table have been checked and are correct.

6. To make it more clear for the reader please clarify what is e.g. “age2” or “bmi2” or “age3” in the signature of Table 3 and ESM Table 2. If this would be e.g. the BMI from previous surveys please explain why used as covariate in a cross-sectional association analysis.
Apologies for the confusion. The 2 and 3 superscripts denote squared and cubed terms. The footnotes have been altered to change these abbreviations to words, e.g. age$^2$ changed to “age squared” and BMI$^3$ changed to “BMI cubed”. These were used as covariates in the analyses where a significant polynomial relationship existed between the outcome phenotype and age or BMI.

Major Compulsory Revisions

7. Actually there are nearly 20 type 2 diabetes risk genetic variants identified. What was the reason just selecting these genes? Please comment on that.
We realise that many more variants with strong statistical claims are emerging and aimed to convey this with the following line in the Introduction:
“Type 2 diabetes (T2D) is a complex disease with numerous risk factors, including a growing number of known genetic susceptibility variants.”

The last sentence of the Introduction gives our rationale for selecting the eight SNPs: “These variants represent those with the strongest reported effects on type 2 diabetes risk and, for the FTO variant, BMI.”

We agree that ideally we should type more variants but we chose those that we were most confident were “real” at the time of the study. We admit that this was slightly subjective. The recent GWAS efforts means the number of possible variants to type has increased dramatically and typing them all would be beyond the scope of this study, although we agree that the results are of importance.

8. To my knowledge there are actually no case-control studies available for the BHS cohort dealing with SNP genotype-phenotype associations regarding T2D and obesity. The SNPs investigated in the paper were previously shown to be associated with the risk of T2D and/or BMI and have been replicated. Due to the efforts which had been made to replicate them in independent cohorts, it would be interesting for the reader if the T2D risk SNPs are also associated with T2D or obesity in general in the present study population at any survey time point. Please, could the authors comment on that and where applicable state it in the Discussion section?

We originally chose not to include the results of association analyses of SNPs with T2D risk due to limited power, as only 271 diabetic individuals were available for analysis. However, it is true that these results for the BHS population are of interest nevertheless, and have therefore included in the revised manuscript cross-sectional association analyses of all of the studied SNPs with the binary outcome ‘history of diabetes’, and of the FTO SNP with the binary outcome ‘obesity’. Paragraph 2 of the Statistical Analysis section has been updated to include the details of these analyses, and the results are given in the new ESM Table 3 and summarised in paragraph 3 of the Cross-sectional Results section. The results are also referred to in paragraphs 2 and 4 of the Discussion.

9. In the total cohort an association of the FTO SNP with BMI was found with a relatively robust p-value of 0.003 but not in the unrelated sample set. Since the FTO SNP is relatively robustly replicated even in smaller sample sets and ~3000 samples seem to be an adequate cohort size what could be the reason for that? Please comment on that and where applicable state it in the Discussion section.

It is true that in cross-sectional analysis of 1994/95 survey data for the unrelated sub-cohort, the FTO SNP rs9939609 was not significantly associated with BMI. This analysis was performed with the aim of estimating the percentage of variance explained by the SNP, though this turned out to not be possible due to the non-significant result. However, for the purpose of assessing the association in the data used for longitudinal analysis, the main effects terms of the longitudinal analysis results may be examined instead. This is a more powerful approach as more data points for the same individuals are considered. In fact, the main effects results of longitudinal analyses do show a significant association between rs9939609 and BMI in the unrelated sub-cohort, though we didn’t originally report them.

We have now made a number of changes to the manuscript to clarify this issue:
1. Cross-sectional results section, paragraph 1: Clarification that the non-significant association was observed at the 1994/95 survey, and statement to indicate the purpose of the analysis.
2. Longitudinal results section, paragraphs 2 and 3: Statement of longitudinal main effects results for the fasting glucose and BMI outcomes.
3. Discussion paragraph 4: Clarification that rs9939609 and BMI were significantly associated in both the whole and unrelated populations.

Though the longitudinal main effects results suggest that a reason for not observing an association in the cross-sectional analysis may be a lack power, it remains possible that the effect size of rs9939609 on BMI is also smaller in our population compared to effect sizes reported for other populations. This is broadly covered in paragraph 5 of our discussion.

Finally, to avoid potential confusion when comparing the reported 0.4kg/m² effect size with our results, we have also added the words “(log units)” to the Statistical Power section of the Methods as a reminder to the reader.
10. As the author state in the discussion section, previous studies suggest that the seven SNPs alter T2D susceptibility through effects on insulin sensitivity or pancreatic beta cell function. Therefore, the study would greatly benefit from including traits such as 30 min glucose or 120 min glucose levels. If such data are available, did the authors check these additcomment on that and discuss it in the paper.

We agree that the results of such analyses would be interesting and important. Unfortunately, although the BHS did take 120 minute glucose readings in 1972 and 1981, there is insufficient data from 1972 to allow a longitudinal analysis. We have added a suggestion for such future studies to the end of the Discussion.

Importantly though, our study does report cross-sectional SNP associations with HOMA-S and HOMA-B (ESM Table 2), indicators of insulin sensitivity and beta-cell function, respectively. We have added a mention of these results to paragraph 2 of the Discussion.

Reviewer 2:

1) At present more than 20 T2D risk loci have been identified. How have the genes been selected for the current analysis?
See response to Reviewer 1, comment 7.

Do these variants show any association with T2D in the BHS (even power is low due to only 271 diabetic subjects)?
See response to Reviewer 1, comment 8.

The authors should include in the discussion for which variants any effects on fasting glucose and/or indices of beta cell function have been reported.
Paragraph 2 of the Discussion reports that rs44022960 has been observed previously to be associated with fasting glucose, and also details which SNPs have been observed previously to be associated with beta cell function. We have added an additional sentence to indicate previous findings for the association of SNPs other than rs44022960 with fasting glucose level.

2) The authors report that there was no significant association of the FTO variant on BMI in the subsample of about 2800 unrelated subjects. Based on the previously reported effect size in the British cohort the detection of an association would be expected.
See response to Reviewer 1, comment 9.

Are the allele frequencies the BHS similar to European samples? It would be helpful to include this in the discussion.
See response to Reviewer 1, comment 4.

Furthermore, based on the fact of missing association in the cross sectional analyses it is not clear if any effects would be expected in the longitudinal analyses? This issue should be shortly discussed.

The following passage has been added to paragraph 3 of the Discussion, following on after discussion of the absence of cross-sectional results for fasting glucose SNPs:

“Associations may also be more difficult to detect if SNP effects vary depending on factors such as other gene variants, lifestyle factors and also age. If a SNP has an age-varying effect on a particular trait, such as an effect to raise fasting glucose level as of middle age for example, then cross-sectional analysis of participants of all ages may miss the association. On the other hand, longitudinal analysis may still identify the relationship, by examining the association of the combination of SNP allele and age with phenotype in aging individuals, through a SNP × age interaction term.”

Furthermore, though our longitudinal analyses were performed on the smaller, unrelated population rather than the whole population used for cross-sectional analyses, the use of multiple measurements for participants increased the power to a similar or higher level, as reported under Statistical Power in the Methods section.
3) The authors report that the whole cohort of 4554 subjects was included in cross-sectional analyses. Please clarify that T2D subjects (6% based on table 1) or subjects with lipid-lowering drugs (2.5% based on table 1) were excluded while assessing glucose and insulin levels or lipids respectively.

Our cross-sectional analyses of the whole cohort of 4554 subjects did not exclude individuals with T2D or those taking lipid-lowering drugs because the removal of individuals from the dataset would have disrupted the pedigree structure required for the QTDT program used for analysis. Instead, a binary covariate, “history of diabetes”, indicating whether or not an individual had ever been diagnosed with T2D was included in analyses of the following outcomes, which were found to be associated with the covariate in cross-sectional generalised linear model analyses of the unrelated sample: fasting glucose level, fasting insulin level, HOMA2-%B, HOMA2-%S, HDL, LDL, DBP. The footnotes of Table 3 and ESM Table 2 indicate that these outcomes were adjusted for the “history of diabetes” covariate.

4) In the legend for ESM Table 2 it is not completely clear what age and BMI 2 or 3 refer to. It would make understanding easier if the authors would clarify these terms.

See response to Reviewer 1, comment 6.

5) Please include a reference for the last sentence on page 3.

References have been added as requested.