Author’s response to reviews

Title: Studies of association of AGPAT6 variants with type 2 diabetes and related metabolic phenotypes in 12,068 Danes

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Author’s response to reviews: see over
Dear editor,

We greatly appreciate the interest in our work, and thank for the constructive criticism and comments given by the 3 referees and. We hope that we have addressed each of the issues raised appropriately, and where requested revised the manuscript accordingly.

Please find our specific answers and comments below:

Reviewer I: Sébastien Robio du Pont

Version: 2 Date: 9 May 2013

Reviewer’s report:
In this manuscript L.S. Snogdal et al. investigated the association of 11 tagging SNPs in the AGPAT6 candidate gene with metabolic traits in Danes populations. The authors did not identify associations that survived Bonferroni corrections and concluded that genetic variation in AGPAT6 does not contribute to obesity/T2D related traits.

Major Compulsory Revisions:
1- Considering the phenotype described in the agpat6 knock-out mouse model, why did the authors investigated T2D-related traits in the study?

RESPONSE: We agree with the referee, that the phenotype of the agpat6-KO mouse model suggests that inactivating mutations in agpat6 protect against diet-induced obesity and other phenotypes normally associated with insulin resistance and T2D. However, the mouse also showed lipodystrophy, which is always associated with ectopic lipid deposition and insulin resistance in humans. In mice, whole-body KO of agpat6 may cause local or systemic compensatory mechanisms, e.g. increased energy expenditure (EE) due to increased browning of fat, stimulation of EE in brown fat, or increased uncoupling of respiration in different tissues. Whole-body KO of other genes, which are crucial in the protection against obesity and insulin resistance, have shown similar paradoxical results due to compensatory mechanisms. For example whole-body KO of PGC-1alpha resulted in paradoxically lean mice, that were resistant to diet-induced obesity (Lin J, Cell 2004;119:121-35). Moreover, the above-mentioned potential compensatory mechanisms may not be seen in humans with inactivating AGPAT variants. On the other hand, some variants may also increase GPAT4 activity. Therefore, given the known importance of GPAT4 in de novo triglyceride synthesis, and, hence, tissue-specific lipid deposition, the reported action of insulin on GPAT4 activity/phosphorylation, as well as the lipodystrophy in agpat6 KO mouse, we still believe that the AGPAT6 gene variants are very interesting in relation to T2D and related traits. We agree that we did not á priori knew whether the AGPAT6 variants protected or increased the risk of T2D and related phenotypic traits. We have therefore changed the phrasing of our hypothesis (page 5, line 6) and similar sentences to indicate that we look for associations and not exclusively whether AGPAT6 variants increase the risk.
2-As the agpat6 knock-out the KO mouse highlights a lean phenotype, it seems relevant to test the association for SNPs with leanness (e.g. people with BMI < 18.5). This study could be done in the total population (12,068 Danes) with appropriate adjustments for T2D status in addition to gender and age in order to maintain sufficient statistical power.

RESPONSE: We only had very few underweight (BMI<18.5) individuals in our cohort. We could possibly do association analyses of underweight people (BMI <18.5) but we did not expect it to add much extra information it was not part of our hypothesis and statistical power in this analysis would be very limited.

3-The analysis of the BMI as a quantitative trait could also be done in the 12,068 individuals adjusting for sex, age and T2D status.

RESPONSE: We did not initially report these analyses in the manuscript, however we have now performed the analyses. We do not find significant associations. These additional analyses have now been mentioned in the revised manuscript (page 8 line 23).

4-In the methods section, “Statistical power”, the authors assess the power of their study for ORs ranging from 1.15 and 1.30 whereas ORs of common variants in the last DIAGRAM meta-analysis are comprised between 1.05 and 1.20. Could they calculate the power for lower ORs and add a table or a graph with these calculations for a range of ORs and allele frequencies.

RESPONSE: Thank you for this comment which we find very relevant. As recommended, we have performed power calculations for more moderate ORs. These analyses have been added as a supplementary figure S1 in the Supplementary files page 2 and have described in the revision (page 8 line 9-18).

5-In the methods section, “Statistics”, the Bonferroni correction does not account for all the statistical tests performed in the study. Please update the manuscript accordingly even if it does not modify the conclusions of your paper (nominally significant results).

RESPONSE: We only corrected for the number of SNPs in our manuscript. We cannot correct for the number of traits by Bonferroni correction, since the traits studied are not independent and Bonferroni correction would therefore be over-conservative.

6-In the methods section, “Statistics”, the authors precise at the end of the Results section that: “adjustment for BMI gave no further information”. Could they add further details?

RESPONSE: We also performed the analysis including BMI as a covariate , however, that did not add any information to the analysis, and results are not shown. This sentence has been added to the statistics section.

7-In the methods section, “Results”, the authors may replicate their nominally significant associations with T2D in publically available GWAS consortium datasets such as DIAGRAM?
RESPONSE: We have now added results for 7 of the 11 examined SNPs, which are available in the newest DIAGRAM results database (Morris et al. 2012 = Ref 24). We have included lookup results for these 7 SNPs in the revised paper (page 8 line 17-18 and page 10 line 12) and included a table showing these results (Supplementary table S2). These results do not substantiate the findings in our population significantly.

8-In the methods section, “Discussion”, second paragraph, the authors argue that the power does not “allow solid conclusions” but in the section “Methods/Statistical Power” they suggest that they have an adequate power, please clarify this apparent contradiction.

RESPONSE: We agree that this is a contradiction. As mentioned above we added extra power calculations as recommended. We corrected the paragraph in the method and statistical power section (page 8 line 17).

Minor Essential Revisions:

In the Abstract:

1-Please mention that the gene has been selected by a candidate gene approach.

RESPONSE: Has been specified in the manuscript (page 3 line 8).

In the methods section, “Participants”, first paragraph:

1-In the Vejle Biobank population the controls were matched for gender and age as mentioned in the manuscript but in the supplementary table S1 the sample differs between cases and controls and the male/female ratio is different. Please clarify.

RESPONSE: Thank you for correcting us. The Vejle Biobank population was only matched for age, and not for gender. The description has been corrected (page 6 line 11).

2-In the supplementary table S1, please check the total sample size and number of man/women. There are some discrepancies regarding the Steno cohort.

RESPONSE: Corrections have been added in table S1 (Additional file_supplementary tables, page 3 line 7).

3-How did the authors check the ethnicity of the participants?

RESPONSE: We appreciate this comment. We don’t have specific information on ethnicity yet all study participants are of Danish nationality. In other studies of the same study material we have by chip genotyping data looked at population outliers and did not observe ethnical population outliers (ref.18) (page 6 line 15-18).

In the methods section, “Participants”, second paragraph:

1-Did the authors include pre-diabetic subjects in the control group?
RESPONSE: No pre-diabetics were included in the study, and this has now been specified in the manuscript (page 6 line 26).

2-Please indicate the T2D inclusion criteria (glucose threshold...).

RESPONSE: We used the WHO1999 criteria. This now has been specified in the paper (page 6 line 12-13).

In the methods section, “SNP selection”:

1-The authors selected the SNPs using HapMap but this database is not design for tagging low frequency variants, it is better to use the 1,000 project for that.

RESPONSE: At the time where we selected our SNPs, the 1,000 project was not yet available online, unfortunately. Otherwise, we completely agree, that it would have been preferable to use the 1,000 project data.

2-Please indicate the rs number of the chose SNPs.

RESPONSE: The rs numbers are the following: rs13252523, rs7357415, rs2977860, rs11785763, rs999188, rs17600159, rs2977845, rs10504041, rs12677439, rs6988044 and rs890220. The rs numbers now been added to the manuscript (page 7 line 8-10).

3-In the legend of fig1, the authors mentioned: “the 11 SNPs is indicating by asterisk” but I checked and I did not see asterisks. Please verify.

RESPONSE: We marked the 11 SNPs with circles in Figure 1 (separate file) instead of asterisks and therefore also corrected the Legend for Figure 1 (page 23 line 4). The edited Figure 1 is attached as a separate file.

In the methods section, “Derived estimates of insulin response and insulin sensitivity from an OGTT”:

1-In addition to the insulinogenic index, HOMA-B may be relevant to assess insulin secretion.

RESPONSE: We did not expect an association with insulin secretion and thus did not perform these analyses.

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:
Reviewer (II): Maggie CY Ng

Version: 2 Date: 26 June 2013

Reviewer's report:
Snogdal and colleagues examined 11 SNPs from a candidate gene, AGPAT6, for association with T2D and related traits in 12068 Danes, including 4638 T2D and 5934 control subjects. No significant associations were found for any traits after multiple comparisons correction, suggesting that genetic polymorphisms at AGPAT6 do not have significant contribution to the studied traits. The paper is well written. While the results are negative for this study of moderate sample size, additional analysis would be useful to refute the test hypothesis.

Minor Essential Revisions
1. AGPAT6 encodes GPAT4 which is involved in triglyceride synthesis, which may in turn associated with obesity and insulin resistance. Since not all diabetic subjects have high triglyceride level and not all subjects with hypertiglycemia will develop diabetes, it may be useful to perform a subset analysis for T2D using T2D subjects with hypertriglyceridemia and control subjects without hypertriglyceridemia to exaggerate the effect, if present.

RESPONSE: We agree that performing such an analysis could potentially strengthen the association with type 2 diabetes yet for several reasons we did not perform this stratification. First, the threshold of hypertriglyceridemia is arbitrary which severely impedes this approach. Second, the subgroups in this subset analysis would be small to merit firm conclusions. Instead we performed population-based analysis of association between SNPs and triglyceride levels.

2. Four variants are nominally associated with T2D which may or may not be in weak LD. It will add depth to the paper by performing haplotype and conditional analyses of these 4 SNPs for assocaition with T2D.

RESPONSE: Since the SNPs we investigate are selected based on tag SNP principles doing haplotype analyses will not increase the derived information from the data. We agree that in the case we had several SNPs in the locus associated with T2D it would be of interest to do conditional analyses to investigate the independence of these signals. Yet in the current data we merely have nominal significant findings and doing conditional analyses will not bring us closer to verify or replicate the putative signals.

Level of interest: An article of limited interest
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
Reviewer (III): Bo Xi

Version: 2 Date: 11 June 2013

Reviewer’s report:
Snogdal et al. investigated the association of AGPAT6 variants with type 2 diabetes and related metabolic phenotypes in Danes. In this study, they genotyped 11 variants of AGPAT6 and tested them in 12,068 individuals. Although non-significant association was found, the statistical power was sufficient to draw the conclusion. Overall, the manuscript is well written and the methodology is sound. The paper is worth publication before addressing the questions below.

Major concerns
1. I suggest the authors additionally adjust for body mass index in case-control study since obesity might be a modifier or confounder. By the way, could the author stratify the data by obese status of subjects (obese vs. non-obese, or overweight vs. normal weight) to see the association between 11 variants and type 2 diabetes in the subgroups? These data may present in appendix files.

RESPONSE: We did perform all analysis with and without adjustments for BMI, age and sex without any significant associations, which have been added as already mentioned above. By dividing the subject in subgroups we had not enough power to conclude if there are significant associations with our SNP’s. Therefore, there are no such data in the supplementary files.

2. Although there was no significant association between each of 11 variants and type 2 diabetes, the combined SNPs may have an effect on the outcome. Thus, I suggest the authors to investigate the effect of total SNPs (sum of 11 variants).

RESPONSE: We acknowledge the idea to perform gene-based combined tests to evaluate the combined impact of the selected SNPs. We are here dealing with common SNPs and not rare variants, and the statistical power to detect association signals in a locus is not increased by doing combined analyses for common SNPs and we have therefore chosen not to do so.

Minor concerns
In line 2, page 6, “four” should be corrected to “five”; in last line, page 21, “, and” should be deleted.

RESPONSE: Corrections have been added to the manuscript.

Level of interest: An article of outstanding merit and interest in its field
Quality of written English: Acceptable
Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests: None