Author's response to reviews

Title: Polymorphisms near EXOC4 and LRGUK on chromosome 7q32 are associated with Type 2 Diabetes and fasting glucose; The NHLBI Family Heart Study

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Author's response to reviews:

Attached please find our revised manuscript entitled “Polymorphisms near EXOC4 and LRGUK on chromosome 7q32 are associated with Type 2 Diabetes and fasting glucose; The NHLBI Family Heart Study” for consideration as a research article in BMC Medical Genetics. Listed below are our responses for all of the reviewers concerns:

Reviewer’s report

Title: Polymorphisms near EXOC4 and LRGUK on chromosome 7q32 are Associated with Type 2 Diabetes and fasting glucose; The NHLBI Family Heart Study

Version: 1 Date: 7 February 2008

Reviewer: Hiroshi Ikegami

Reviewer's report:

This manuscript demonstrated that polymorphisms located nearby EXOC4 and LRGUK genes are associated with susceptibility to type 2 diabetes. The region on chromosome 7q22-36 is linked to obesity related traits in the family heart study.

Comment 1: Please describe about control subjects more precisely:
How were they recruited? How about family history of diabetes? Are they normal glucose tolerance?
Response 1:
The controls used were participants recruited as part of the Family Heart Study who did not report a diagnosis of diabetes. Recruitment for the Family Heart Study was described on page 4. Family history of diabetes was not used as either inclusion or exclusion criteria for either cases or controls. Glucose tolerance testing was not performed as part of the clinical examination for the Family Heart Study. Participants were asked to fast before their examination, and fasting glucose levels were measured from blood. Thus they were not necessarily normal glucose tolerance, but they were neither diagnosed nor treated for diabetes at that time.

We have clarified the description of the recruitment of controls by adding the sentence "The controls used were participants recruited as part of FHS who did not report a diagnosis of diabetes." was added to page 5 of the manuscript.

Comment 2: Although authors mentioned that subjects with non-diabetics with homozygous deletion genotype showed significantly lower fasting plasma glucose than those with insertion allele, but no actual data were presented in this manuscript.

Response 2: Table 4 lists the association results for the fasting glucose levels. Within this table, the beta-estimates provide the difference in mean glucose levels in mg/dl. In addition, on page 9 of the Results, section we describe the decline in glucose levels in mg/dL as follows: "Men homozygous for the deletion polymorphism had a statistically significant decrease in fasting glucose levels (p = 0.038, \( \beta \)-estimate = -2.57 mg/dL) while the TCF7L2 SNP had a modest increase in fasting glucose levels (p = 0.089, \( \beta \)-estimate = 1.91 mg/dL). Neither polymorphism had a significant effect on fasting glucose in women."

Comment 3: Since deletion polymorphism is located on chromosome 7q32, authors should also present the data about metabolic syndrome and obesity-related traits of non-diabetic subjects with homozygous deletion genotype: e.g. BMI, waist circumference, TG, HDL-cho, blood pressure, and fasting insulin level.

Response 3:
We had an a priori reason to study diabetes because of the deletion\( \beta \)-s close proximity to the Exoc4 gene, which has a strong biological rational for influencing glucose levels and therefore diabetes risk. We sought to clarify we are studying diabetes as opposed to a broad class of possibly traits in the 2nd paragraph in the "Conclusion" section of the manuscript (first paragraph of page 11). Specifically we state:

"The region implicated by the deletion and SNP polymorphisms reported here is located between the genes EXOC4 (NM_021807) and LRGUK (NM_144648). Interestingly, EXOC4 is a large gene and its product is part of the exocyst complex 70 (Exo70) that assembles at the plasma membrane of adipocytes in response to insulin and has been reported to play a role in docking and tethering
the glucose transporter 4 (GLUT4) vesicle to the plasma membrane [36, 37]. GLUT4 accounts for much of the insulin-stimulated glucose transport in muscle and adipose tissue [37, 38].

And to add this sentence: “The potential for this gene to influence diabetes and glucose levels prompted us to evaluate the association to these traits.” We are reluctant to extend the numbers of metabolic related phenotypes, as it raises the issue of data mining for results. In this study, we let the biological rationale of the genes that were in linkage disequilibrium with the deletion guide us in deciding which phenotypes to test instead of starting a “fishing expedition” to find a significant result.

Reviewer’s report

Title: Polymorphisms near EXOC4 and LRGUK on chromosome 7q32 are associated with Type 2 Diabetes and fasting glucose; The NHLBI Family Heart Study

Version: 1 Date: 8 February 2008

Reviewer: Martine Vaxillaire

Reviewer’s report:

The paper by Laramie et al. reports an interesting study focusing on the genetic variation (deletion and SNP polymorphisms) in a 1.08 Mb region on chromosome 7q32 which was shown to be linked to metabolic syndrome and obesity in the Family Heart Study. The study design and the methodology used are correct, albeit a very limited statistical power due to the low number of diabetic patients (207 diabetics) included in the genetic association analysis.

Major Compulsory Revisions:

Comment 1: One more general concern with this study is that the original linkage was obtained for another disease status, and particularly obesity related traits, and there is no attempt to replicate the present findings in other cohorts of patients outside the FHS participants.

Response 1: Linkage analyses in the Family Heart Study have been performed for a variety of quantitative traits, but because recruitment was not focused on a particular disease, there are limited numbers of affected-sibling pairs to perform linkage to disease traits. Linkage to diabetes has not been evaluated in this region, but the two quantitative traits linked to the region were BMI and the metabolic syndrome factor score.

We agree with the reviewer’s comment that we had not emphasized the original linkage to Metabolic Syndrome, and we have highlighted that result on page 4, where we have added the sentence “Importantly, linkage to metabolic syndrome has also been reported for this cohort in the 7q32 region [33].”

Comment 2: and there is no attempt to replicate the present findings in other cohorts of patients outside the FHS participants.

Response 2: We modified the Conclusion, to emphasize the findings in other
cohorts (see Conclusion; pages 11 and 12 paragraph 3):

Findings in other cohorts also support the presence of a gene(s) influencing diabetes risk in this region. Genome-wide associations for diabetes were recently performed by the Wellcome Trust Case Control Consortium [40] and the Diabetes Genetics Initiative (DGI) [41], and the results are publicly available. We examined association results for the SNPs in the region spanning the EXOC4 gene, the deletion, and the LRGUK gene, which included 202 SNPs in the Wellcome Trust and 128 SNPs in the DGI. From the Wellcome Trust results, we identified 37 SNPs with association p-values less than 0.05, and from the DGI, we identified ten SNPs with association p-values less than 0.05. The best p-value identified in the Wellcome Trust results in this region was 0.0004 at rs6963221 in EXOC4. In the DGI results, the best p-value was 0.015 at rs17167492 in LRGUK. These results from two independent samples lend support for polymorphisms in the region influencing diabetes risk.

These independent populations, these association results support to our findings that this region influences diabetes risk.

Comment 3: Moreover, T2D in this study sample was defined based on participants self report (as well as BMI at age 25). Probably, all these limitations are reflected by the rather moderate association found between T2D and the TCF7L2 SNP, the one which was largely and reproducibly replicated in most of the reported association studies. Thus, the sentence in the abstract and in the discussion, where it is stated that the evidence of association to T2D for the deletion is comparable to that of TCF7L2 SNP, is somewhat ambiguous and should be changed.

Response 3: We agree with the reviewers comment and in the abstract we have removed the sentence:

In this sample, the magnitude of the association to T2D, albeit protective, was comparable to that seen for the TCF7L2 polymorphism.

Comment 4: One interesting finding is that the non-diabetic male homozygous deletion carriers may have lower fasting glucose levels, though the polymorphism does not seem to overlap with the EXOC4 gene, but is located downstream of that gene and just upstream of the LRGUK gene. There is no strong evidence for a functional effect of the deletion polymorphism; and to my view, in this paper it is difficult to make a link between the deletion polymorphism and the other SNPs tested, and more importantly with the putatively associated SNPs: in the abstract (but not in the Results section), it is reported that six SNPs are significantly associated to diabetes. This needs to be improved in the current report.

Response 4: We agree with the reviewer that these SNPs should be listed in the results section, and the following sentence has been added to page 9:

Seven SNPs (rs3823572, rs12531707, rs11770757, rs7457999, rs6953590, rs12670589, and rs1421483) demonstrated significant association (p < 0.01) to fasting glucose.
Additionally, setting a significance threshold of \( p \leq 0.01 \) resulted in seven SNPs showing association to diabetes. These results have been updated in the Abstract, Methods and Results section. Table 5 has been revised to change the SNP names to bold font to designate statistical significance to make it easier to identify those SNPs and to show their physical proximity to the deletion. Finally, we would like to point to Figure 2 that shows the LD \((r^2)\) relationship between the SNPs and the deletion. The evidence for LD supports the link between the deletion and the polymorphism in association to diabetes.

Specific points:

Comment 5: the deletion polymorphism has to be clearly indicated (sequence and number of nucleotides) in the text of the manuscript.

Response 5: We have carried out numerous molecular biology studies to define the deletion boundaries. Currently, we believe that the deletion is a different size for different individuals. Therefore, we have not been successful in pin-pointing the deletion boundaries in each individual or a range of boundaries in the general FHS study population but it is still under active investigation in our laboratory.

Comment 6: does the description of the genotype cluster assignment (in page 5) mean that about 30% of genotypes are missing for the deletion polymorphism?

Response 6: We have clarified our statement on Page 6 paragraph 1 under ¿Deletion Detection¿ which indicates that only 69 individuals were not able to be genotyped, which represents 2.8% of the total population for which deletion genotyping was attempted.

The modified sentence is:

¿A small number of individuals whose ¿Ct value fell outside of the three genotype clusters (n=69, 2.8%) were coded as missing genotypes.¿

Comment 7: In the results for SNP association: the number of polymorphisms with evidence of association should be indicated in the text on page 9. Indeed, from the Table 5, we can not easily identified which are the six SNPs (as reported in the Abstract). Please clarify this point.

Response 7: We agree with the reviewer and the necessary changes have been made. Please see Response 4 for details.

Comment 8: the p-values given throughout the manuscript should be as nominal p-values, as no correction for multiple testing (number of polymorphisms and number of statistical tests) was made.

Response 8: We agree with the reviewer and a sentence has been added to the Methods section on page 7 that states:

¿No correction for multiple testing was used in these analyses and, therefore, all p-values are reported as nominal p-values. ¿
The following lists all of the changes to the original manuscript:

Page 2: Six was changed to Seven in the second paragraph of the Abstract
Page 2: The sentence, ¿In this sample, the magnitude of the association to T2D, albeit protective, was comparable to that seen for the TCF7L2 polymorphism.¿ was deleted
Page 3: The last sentence in 3rd paragraph of the Background section was changed to read, ¿Importantly, linkage to metabolic syndrome has also been reported for the Family Heart Study cohort in the 7q32 region [33].¿ and a reference was added.
Page 5: A sentence was added in the ¿Subjects¿ subsection of the Methods section that reads, ¿The controls used were participants recruited as part of FHS who did not report a diagnosis of diabetes.¿
Page 6: The phrase, ¿A small number of¿ was added to the last sentence of the ¿Deletion Detection subsection in the Methods section.
Page 6: The phrase, ¿the SNPs¿ was replaced by, ¿polymorphisms¿ in the first sentence of the ¿Statistical Analysis¿ subsection of the Methods section.
Page 7: The sentence, ¿No correction for multiple testing was used in these analyses and, therefore, all p-values are reported as nominal p-values.¿ And the sentence, ¿The total study population was analyzed, analyses stratified by sex were performed.¿ In the subsection ¿Statistical Analysis¿ in the Methods section.
Page 9: The sentence, ¿Seven SNPs (rs3823572, rs12531707, rs11770757, rs7457999, rs6953590, rs12670589, and rs1421483) demonstrated significant association (p ¿ 0.01) to fasting glucose.¿ was added to the subsection ¿SNP association¿ within the results section.

For consistency, the words p-value was changed to ¿p¿ throughout the manuscript.

All authors have reviewed and approved the manuscript for resubmission. The material has not been submitted elsewhere. The authors have no conflicts of interest to disclose.

Thank you for reconsidering this article for publication in BMC Medical Genetics.

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