Author's response to reviews

Title: A tagging SNP in INSIG2 is associated with obesity-related phenotypes among Samoans

Authors:

Ranjan Deka (ranjan.deka@uc.edu)
Ling Xu (josephinexu1983@gmail.com)
Prodipto Pal (prod.pal@gmail.com)
Palanitina T Toelupe (ceo@health.gov.ws)
Tuiasina S Laumoli (tuiasinasl@americansamoa.gov)
Huifeng Xi (xihuifeng@gmail.com)
Ge Zhang (zhangge.uc@gmail.com)
Daniel E Weeks (weeks+@pitt.edu)
Stephen T McGarvey (Stephen_McGarvey@brown.edu)

Version: 2 Date: 20 July 2009

Author's response to reviews: see over
Dear Editor-in-Chief,

Please find enclosed the revised manuscript referenced above. The reviews have been very helpful for improving the quality of the manuscript, and we would like to thank the reviewers for their time and effort in providing their comments. We have organized our responses to the reviewers’ comments as noted below. The changes in the manuscript are highlighted in ‘red’ color. We hope with the changes, the manuscript will be acceptable for publication in BMC Medical Genetics.

Thank you for your consideration.

Sincerely,

Ranjan Deka, Ph.D. on behalf of all authors

Response to the Reviewers:

Reviewer: Dr. Partha Majumder

Comment: The study selected 4 tagSNPs from the CHB database; rs7566605 turned out to be located on the extremal 5’ end. This, I think, is a major limitation of this study. The authors should have selected tSNPs from a region in and around the INSIG2 gene, such that rs7566605 was enveloped by tSNPs on both 5’ and 3’ ends. (Currently, there are no SNPs in the 5’ end of rs7566605.) The present study has found association with rs9308762, which is on the opposite end (3’ extremal end), in subsets of the total sample. I think the authors should select some SNPs from the region 5’ to rs7566605 and test for association.

Response: We appreciate this pertinent comment from the reviewer. However, we would like to state that rs7566605 is located ~10kb upstream of the INSIG2 gene. Further, this SNP tags common variants up to ~10kb in its upstream region. So we think it covers up to ~20kb upstream of the INSIG2 gene. In almost all follow-up studies cited in the manuscript, this is the only SNP analyzed. Our study thus not only covers an extended region, but also directly interrogates this SNP for replication or lack thereof in the Samoan population.

Comment: On page 3 it is stated “the Samoans from both territories form a single socio-cultural unit with frequent exchange of mates and genetically they represent a single
homogenous population.” I wonder whether the authors have actually tested for population substructuring. If so, this should be mentioned.

Response: In support of this statement, we had cited our published papers [16,17]. We had also done a formal analysis of substructure. To reinforce the statement, we have now added the following at the end of the sentence on page 3: “without any evidence for significant population substructure as supported by our prior genetic analyses” and added another reference (Tsai et al. 2004, new reference number 18).

Comment: On page 3 it is further stated “This study sample was restricted to those 907 participants with a fasting glucose level of <=110mg/dL in order to reduce the confounding of hyperglycemia and type 2 diabetes on the obesity traits.” However it is not stated whether any of the 907 participants on anti-diabetic medication? This is important.

Response: This is a very important point. We re-reviewed our entire data set and found only two of the 907 subjects were on anti-diabetic medication. We have added a sentence in the manuscript (page 3) to clarify this. We believe this will not impact on the results of our analysis.

Comment: While the findings of this study are interesting, these are not exciting. I do not think that the findings of this study have provided clear confirmatory evidence of association of rs7566605 with obesity among Samoans. I suggest that the authors carry out some further work to increase the strength of this study.

Response: We do agree that the findings may not be exciting. We would like to note that we conducted the study in a unique population – the levels of overweight and obesity are remarkably high in the Samoans; and also, the LD levels are higher compared to other outbred populations. Importantly, our study while not confirming the association of rs7566605, the findings do not rule out the involvement of the INSIG2 gene in obesity among the Samoan population. Albeit a study with larger number of markers will increase the strength of the findings. However, the purpose of this study is to perform a comprehensive analysis using tagging SNPs, and as we have stated in the manuscript that the observations at this stage are preliminary.

Reviewer: Dr. Christoph Lange

Comments: Very interesting ms. Well written stats analysis without any flaws. An article of importance in its field.

Response: We appreciate the comments of Dr. Lange.

Reviewer: Dr. Christian Dina

Comment: This concise article does not, in its present form, add much to the question of whether INSIG2 region is associated with obesity or not and the results should be presented in perspective with previous results both in European and Asian samples.

Response: This is a helpful comment – particularly in the context of our study population, results from other Asian populations are very relevant. Now, we have added a sentence in the final paragraph of the Results and Discussion section on page 5 highlighting the published studies of association of rs7566605 with obesity in Asian populations and cited those publications (new Reference numbers 27-31).
**Major Revision**

**Comment:** What is the difference between LD in Samoans and Europeans? Does it explain why another SNP than the initial one would be found (marginally) associated in obesity?

**Response:** This is a very pertinent question in the context of our results. We had previously shown that LD in the Samoans throughout the genome extends over a larger region than the Europeans. We have now added the following sentence in the final paragraph of the Results and Discussion section to highlight this observation. *It should be noted that using both genome-wide microsatellite markers at a spacing of 10cM and over 7,000 SNPs distributed on chromosome 21, we observed a significantly higher level of LD in the Samoans compared to the European populations [18,22] favoring the identification of a surrogate marker further away from the true variant in the relatively isolated Samoan population.*

**Comment:** The article would gain in interest if the results of present study were compared with results in other Asian populations.

**Response:** Please see our response to the comment above.

**Comment:** The result is less conclusive than what is stated. A p of 0.009 just means that we need more samples. The authors should change a little bit their conclusion.

**Response:** We appreciate this comment. From the perspective of genome-wide association studies, extremely small p-values are needed due to multiple testing issues incurred by testing large numbers of SNPs. However, here we are testing a specific gene with a limited number of markers in a replication framework. The level of evidence needed in this context is much more modest than that needed in a genome-wide association study.

**Comment:** The association was found under recessive model only, even in the original study. Therefore, this genetic model (instead of or along with the additive) should be explored and presented.

**Response:** We had tested association under three different genetic models. Under recessive model also, we found similar results (for the total Samoan population, BMI, \( p = 0.038 \); ABDCIR, \( p = 0.018 \)). In Table 2, we have reported the results of association under the additive model because of its simple focus for detecting near-additive risks. We have, however, added a sentence in the Results and Discussion section (page 4) as: *These results are similar when the test statistic was performed under the recessive model, with both BMI (\( p = 0.038 \)) and ABDCIR (\( p = 0.018 \)) showing association with rs9308762.*

**Minor**

**Comment:** I do not understand the permutation procedure: is it SNPs specific or does it apply to the 5 tests simultaneously? If the last is true, why using Bonferroni? It is not clear why presenting separate results for males and females for INSIG 2 gene. A quick review of the literature does not point out any differential effect.

**Response:** We used permutation test to adjust for the four SNPs but not for the five tests among the five different groups. As stated in the text, ‘A stringent multiple testing correction for this would be to use the Bonferroni correction, which would require testing at the 0.05/5 = 0.01 level.’ However, since the test results among the five groups are highly correlated, the use of Bonferroni would be over-conservative.
On the second issue of presenting separate results for males and females, since the traits have gender effects, we regarded it to be important to present the results separately even though no differential effects were seen in other studies.

**Comment:** Please display the p-value, OR and confidence interval of the SNPs presented as significant in the main test. It is a bit difficult switch text/table when we read the main text.

**Response:** We apologize for this omission and have now provided the p-values in the text.