Author's response to reviews

Title: The INSIG2 rs7566605 genetic variant does not play a major role in obesity in a sample of 24,722 individuals from four cohorts

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Re: MS5875181992362191  

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To Whom It May Concern:  

We are submitting a revised manuscript entitled “The INSIG2 rs7566605 genetic variant does not play a major role in obesity in a sample of 24,722 individuals from four cohorts”. We would like to thank the reviewers for their many helpful suggestions, and we have indicated our responses to the referees’ comments below organized by reviewer. 

Reviewer: Kiminori Yamane  

Major Compulsory Revisions:  

1. The authors concluded that the INSIG2 SNP was not related with obesity in Abstracts and Conclusions. However, the white ARIC study participants with CC genotype had lower waist-to-hip ratio compared to those with the G allele. In addition, the similar result was found in African-American ARIC study subjects. Waist-to-hip ratio is known to be one of the indices for obesity. Authors should assess the discordance.  

In the white ARIC study participants, individuals with the CC genotype had a lower waist-to-hip ratio when compared to those carrying a G allele, while the African-Americans with the same genotype had a higher waist-to-hip ratio. It is our interpretation that given the nominal p-values for both of these associations that were not corrected for multiple testing, and the difference in the direction of these associations for the two ethnic groups, caution is required when asserting that this represents a true positive association. In addition, a combined analysis of all study participants adjusted for age, gender, race, and study did not show a significant association between the INSIG2 rs7566605 variant and waist-to-hip ratio (page 15, lines 10-20). However, we have changed the title of our submitted manuscript from “A polymorphism in the INSIG2 gene is not associated with obesity in a sample of 24,722 individuals from four cohorts” to “The INSIG2 rs7566605 genetic variant does not play a major role in obesity in a sample of 24,722 individuals from four cohorts” to respond to this concern.  

2. There were no significant differences in BMI, waist girth, or prevalence of obesity according to the genotype in each population. The authors should add comment on the power to detect association between these parameters and this SNP. Interpretation might be altered if not enough power is the reason for not finding a significant difference in these parameters.
Power calculations have been performed for these parameters, and have been described in the manuscript in the methods (page 11, line 19 – page 12, line 1) and discussion sections (page16, lines 7-16). There was adequate power (>80%) to detect an effect of the size previously reported (OR= 1.29 – 1.75) for the association between rs7566605 and BMI considered as a dichotomous variable (BMI > 30 kg/m$^2$) for all racial groups in the ARIC and GENOA cohorts, while a slightly larger odds ratio is detectable for both whites and African-Americans in the CARDIA cohort. There was 95% power to observe a small effect (i.e., $R^2$ < 1%) of the INSIG2 sequence variant for the minor allele frequencies observed for each study population after stratification by race.

3. The first research by Herbert et al. (Science 2006, 312:279-283) suggested that the significant relationship between the SNP and obesity was confirmed in four out of five additional cohorts, as authors also described. Authors should discuss the considerable reason why there was difference between the previous studies and present study.

There are a number of possible reasons to explain the different associations detected between the INSIG2 variant and obesity in different populations. These include differences in ascertainment, study design, genotype call rate, the degree of LD between rs7566605 and a true causative variant, and heterogeneity between the populations due to different genetic or environmental factors. These have been noted in the discussion section of the manuscript (page 15, line 22 – page 16, line 3).

Minor Essential Revisions:

1. Some SNP number was stated as “rs756605”. This should be converted into “rs7566605”.

   The SNP number has been corrected to rs7566605 throughout the manuscript.

2. Some Mexican-Americans subjects in GENOA study were recruited that contained at least two siblings diagnosed as having type 2 diabetes. Diabetes might affect the body weight and body composition. Authors should consider the influence or the limitation.

   Obesity is a known risk factor for diabetes in Mexican-Americans in south Texas, so that many individuals will have both diabetes and obesity. There was a high percentage of obese individuals in this study group, and the average BMI exceed 30 kg/m$^2$. As noted in the discussion (page 14, lines 1-14), there is no consistent pattern when considering previously published studies for the association between obesity and the INSIG2 rs7566606 variant, with failure to detect an association in a number of studies where the average BMI observed in the population was $\geq$ 30 kg/m$^2$.

3. In GENOA study, final study sample (4766 participants including 1,731 African-Americans, 1,421 white, and 1,614 Hispanic) was likely to be larger than initially recruited sample (1,228 African-Americans, 1,022 Non-Hispanic white, and 954 Mexican-Americans). This was the result that it was hard to understand. Perhaps,
“additional siblings without disease (page 9, line 14)” might be included. Authors should make it clear.

The statement that additional siblings were invited to participate in GENOA has been added in the methods section (page 9, line 12).

4. Genotyping success rate was not perfect. How many times was the SNP determined by the system? Did authors perform direct sequence method for the determination of the SNP?

Additional information about the genotyping success rate has been added to the methods section (page 10, line 23 – page 11, line 1). The SNP was genotyped only once for each individual in the study populations, and the SNP was not directly sequenced to determine the INSIG2 rs7566606 genotype.

5. Authors suggested as follows (page 13, line 2), “The initial association…but not all cohorts (28-36)”. The reference number 37 (Oki et al. Br. J. Nut 2008) also appears to report negative association between the SNP and obesity

The reference Oki K et al. Br J Nutr 101:322-327, 2009 has been grouped with other references reporting a negative association between rs7566605 and obesity (page 13, lines 9-11).

Reviewer: Camilla Andreasen

Major revision:

Introduction:

The focus of the introduction needs a change.

A general introduction describing the public health significance of obesity has been added at the beginning of the introduction (page 5, lines 2-15).

The study groups for the present study should not be described in the introduction (page 5 line 14 to page 6 line 5) – it is done in the methods section.

The study groups are no longer described in the introduction.

The very detailed description of INSIG2 function (page 6 line 13 to page 7 line 6) is redundant. When the aim of the study is to perform association studies, it would be much more interesting to describe which association studies have been made on this genetic variant previously. All abbreviations made in the introduction are only used one time and therefore unnecessary.
The detailed discussion of the INSIG2 function has been reduced but not eliminated since we feel it is important to assess the plausibility of the gene as a determinant of obesity (page 6, line 13 – page 7, line 2). We have added additional information about previously published association studies in the discussion (page 13, line 11 – page 15, line 8). All abbreviations that were used only once and included in the introduction have been removed.

Methods:

How is race determined? – self-reported? This should be clearly stated.

Race is self-reported, and this is now clearly stated in the methods section (page 12, lines 1-2).

Statistical analysis:

Sibship = a pair of siblings?

A sibship is at least a pair of siblings that were recruited from each family enrolled in the GENOA study. Any additional siblings in the family were also invited to participate. These details concerning ascertainment are now included in the part of the methods section describing the GENOA study (page 9, lines 1-14).

Which statistical methods are used when analyzing the GENOA study? How is it taken into consideration that siblings, which share much genetics, are analysed?

Before conducting the statistical analyses in the GENOA study participants stratified by race, we chose one sibling at random from each pedigree to assess the association between the \textit{INSIG2} variant and obesity. When the results were compared with those obtained using all participants we found that they were similar. We therefore decided to include all study participants in our report. This information is now included in the methods section of the manuscript (page 11, lines 15-19).

Please state what adjustments that are inferred in the analyses of anthropometric measures – it is perfunctory to just give a reference.

All statistical analyses of the association between anthropometric measurements and rs7566605 were adjusted for age and gender. This is now included both in the relevant tables and in the methods section where the statistical analyses are described (page 11, lines 11-12, page 11, lines 13-15).

Why is the homogeneity between studies analysed – is the combined study material studied at all?

We provided a combined p-value for all studies using the Mantel-Haenszel method for BMI modeled as a dichotomous variable (BMI > 30 kg/m$^2$). This is more clearly
Please perform power analyses – what effect sizes is expected to, are there sufficient statistical power to detect this when the study sample is divided into smaller subsamples.

Power calculations have been performed for the individual subsamples. There was adequate power (>80%) to detect an effect of the size previously reported (OR= 1.29 – 1.75) for the association between rs7566605 and BMI considered as a dichotomous variable (BMI > 30 kg/m^2) for all racial groups in the ARIC and GENOA cohorts, while a slightly larger odds ratio is detectable for both whites and African-Americans in the CARDIA cohort. There was 95% power to observe a small effect (i.e., R^2 < 1%) of the INSIG2 sequence variant for the minor allele frequencies observed for each study population after stratification by race. This information is now included in the methods (page 11, line 19 – page 12, line 1) and discussion sections (page16, lines 7-16) of the manuscript.

Consider making meta-analyses between same ethnicities from different study groups if homogeneity allows this – this will increase statistical power.

We provided a combined p-value for all studies using the Mantel-Haenszel method for BMI modeled as a dichotomous variable (BMI > 30 kg/m^2). This is more clearly outlined in the statistical analysis portion of the methods section (page 11, lines 12-13), and is also included in the results section (page 12, lines 10-14), and on Table 2. We also combined all study participants for analysis of the obesity-related quantitative traits and adjusted for age, gender, race, and study. This information is now included in the results (page 12, lines 19-21) and discussion sections of the manuscript (page 15, lines 18-20), and on Table 2 and Table 3.

Results:

Why is only baseline data analyzed, it is not exploited at all that you have longitudinal study groups.

Only baseline data was analyzed because of prior arrangements for data distribution from the various cohorts.

Only BMI, weight, waist and waist-to-hip is analysed, are there other phenotypes like total cholesterol, LDL-cholesterol and HDL-cholesterol. Are the endpoint of the different study groups analysed?
Similarly, only anthropometric measurements were analyzed here because of the limited scope of the agreements made for data distribution for the various study groups.

Discussion:

Do not start your discussion with a summary of the literature, state your own results and put them into greater context of results in the literature.

The order of presentation of the results for the study we are reporting here, and results from the published literature has been reversed.

How is your racial results compared to previous studies – discuss your references 2, 25-27 + 28-36 in more depth than just one line. In page 12 line 2-11 you mention a study of fatty acids, triglyceride and cholesterol but you cannot put your own results into a greater context, why not discuss previous studies on obesity measures instead.

References describing associations in non-European study populations have been discussed in greater depth in the discussion (page 14, line 16 – page 15, line 8) and the description of the study of fatty acids, triglyceride, and cholesterol has been removed.

Conclusion:

Skip the summary and state your conclusion.

The summary has been omitted and only the conclusion has been presented.

Tables:

The OR in table 2 does it not refer to the risk of being obese – which is analysed in table 1.

The OR in Table 2 refers to the risk of being obese. The p-value presented in Table 1 is for the difference in rs7566605 allele frequencies between obese and non-obese participants for each cohort.

Minor revision:

A “6” is missing every time the rs-number is used in the abstract.

The rs-number of the INSIG2 variant has been corrected in the abstract.

It is implicit an rs-number is a genetic variant, so the term rs7566606 SNP is wrong to use, the same for INSIG2 gene – it is implicit when it is with capital letters and italic that it is a gene.
The phrases “rs7566606 SNP” and “INSIG2 gene” have been eliminated from the manuscript.

Spaces are missing in several places.

The errors in spacing have been addressed.

Use more commas, the text is difficult to read.

Commas have been introduced in the text to improve clarity and flow of language.

Reviewer: Kikuko Hotta

Minor comments:

1. The relative risk of each genetic variation for obesity seems to be relatively small. Thus, statistical power of this study is better to be calculated.

   Power calculations have been performed, and are included in the methods (page 11, line 19 – page 12, line 1) and discussion sections (page 16, lines 7-16) of the revised manuscript. The power reached 80% to detect an effect of the size previously reported (OR= 1.29 – 1.75) for the association between rs7566605 and BMI considered as a dichotomous variable (BMI > 30 kg/m²) for all racial groups in the ARIC and GENOA cohorts, while a slightly larger odds ratio is detectable for both whites and African-Americans in the CARDIA cohort. There was 95% power to observe a small effect (i.e., \( R^2 \leq 1\% \)) of the INSIG2 sequence variant for the minor allele frequencies observed for each study population after stratification by race.

2. The results of combined analyses should be provided in Table 2 and Table 3.

   The results of combined analyses have been added to Table 2 and Table 3.

Please feel free to contact me if you have any questions or require any additional information.

Sincerely,

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