Author's response to reviews

Title: The obesity-risk variant of FTO is inversely related with the cautious, low-appetite, and slim "So-Eum" constitutional type: Genome-wide association and replication analyses

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Author's response to reviews: see over
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Tom Rowles  
Executive Editor  
BMC Complementary and Alternative Medicine

Dear Dr. Rowles:

Manuscript ID: 1521119315127933

We would like to thank you and the reviewers for taking the time to review our article entitled “The obesity-risk variant of FTO is inversely related with the So-Eum constitutional type: Genome-wide association and replication analyses,” which we would like to resubmit for publication in BMC Complementary and Alternative Medicine as a Research Article.

We have made corrections and added clarifications in the manuscript to address the reviewer’s comments and have highlighted the relevant revisions in yellow in the revised document. In the following pages, you find our responses to the points raised by the reviewers.

We hope that the revised manuscript meets the requirements of your journal for publication, and are looking forward to hearing from you again.

Sincerely,

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Responses to the reviewers’ comments

Reviewer 1 (Dr. Yufang Pei)

Sasang constitutional (SC) medicine categorizes human beings into four types (TE, SE, SY and TY). In this study, Cha et al. defined SC types on the basis of tertiles of SCAT probability values and performed GWA analysis and followed replication analysis for three SC types (TE, SE and SY). They found that the obesity-risk variants of FTO were inversely related with the SE type. The results are interesting. I have several comments:

- Major Compulsory Revisions

  Comment 1: What is the correlation between different SC types? Is there any overlap between any two different SC types?

  Our response:
  We indeed found overlap between the SE and SY types, indicating a correlation between the SE and SY types (Additional Figure 1). As a result, we could not use a single regression model with a multinomial variable (TE, SE, and SY). Please also refer to the comment 2 of Responses to Reviewer 3.

  Comment 2: The authors only performed association analysis for the genotyped SNPs in this study. Genotype imputation with the 1000 Genomes reference panels can infer the genotypes at untyped loci. The imputation algorithm should be used, at least for the FTO gene.

  Our response:
  In the revised manuscript, we have included imputation of SNPs on chromosome 16 with the JPT/CHB HapMap reference panel (HapMap release 22) for association analysis. The imputed SNP data as well as original Affymetrix SNP array data were provided by Korea Center for Disease Control. We can analyze the genotype data only on an offline PC located in Korea Center for Disease Control due to the distribution policy; therefore, we could unfortunately not use the 1000 Genomes reference panels for imputation.

  We have added a paragraph describing the SNP imputation procedure in Materials and Methods section on page 10, and revised second paragraph of Genotyping section of the Materials and Methods section on page 8 by including the information about genotyping rs7185735. After analysis of the association with the SE type, the primary SNP was no longer rs7193144; it was found to be rs7185735. The association trends of rs7185735 were similar to those of rs7193144 because of the strong linkage disequilibrium between the two loci, except for a weak, non-significant association after adjusting for BMI. Therefore, we maintained the main conclusions for rs7193144, instead of for rs7185735. We have also included the results of the SNP imputation in chromosome 16 section in the Results and Discussion sections (pages 13 and 14, respectively). In addition, we have rewritten the first two paragraphs of
Discussion section in the original manuscript into three paragraphs in the revised manuscript due to a logical meaning.

**Comment 3:** The authors did not describe what kind of random-effects model they used and they did not report the results for heterogeneity test. More details should be provided by the authors.

**Our response:**
A random-effects model estimated by the DerSimonian and Laird method was used in the study, as indicated in the Materials and Methods section on page 10. We also added the results of a heterogeneity test (Q-value, $p$, and $I^2$) in Table 2. The heterogeneity test suggested that the SC type data from two populations was homogenous, but we used a random-effects rather than a fixed-effects model, because the recruitment procedures and regions differed between populations.

**Comment 4:** The authors performed association analyses for three SC types. And thus the genome-wide significance at the Bonferroni-corrected level should be 0.05/311944/3.

**Our response:**
After the modified Bonferroni correction ($0.05/311944/3 = 5.34E-08$), there were no significant associations. Therefore, we estimated the significant associations not by Bonferroni-correction but by repeated associations both in KoGES ($<5.0E10-06$) and KCMS populations ($<0.05$). We changed the description of the determination of the significance level in Statistical analysis section of the Materials and Methods section as follows (pages 9 and 10): for GWAS in the second paragraph, “A cut-off $p$-value was $5.0 \times 10^{-6}$.”; for replication analysis in the third paragraph, “A cut-off $p$-value was 0.05.”; and for combined analysis analysis in the last paragraph, “The significance level of SNP in combined analysis was considered as repeated associations in both KoGES ($p < 5.0 \times 10^{-6}$) and KCMS ($p < 0.05$).”

**Reviewer 2 (Dr. Yun Qian)**

**- Major Compulsory Revisions**

**Comment 1:** In Abstract, the authors stated “Therefore, the obesity-risk variant of FTO might be involved not only in body mass increase but also in the determination of body constitution type.” This statement does not seem to be appropriate. The study didn’t analyze the relationship between the genetic variants in FTO gene and body mass increase at all. So it is not suitable to draw this conclusion.

**Our response:**
We have rewritten the above-mentioned sentence in the abstract as: “Therefore, the obesity-risk FTO variant associated with body mass increase might be involved in the
determination of the body constitution type”

Comment 2: Are there any differences between SE and NSE in characteristics, such as age, gender, BMI, waist circumference, fasting blood glucose, etc? Between TE and NTE? As FTO gene related to diabetes has been reported, the authors should consider the potential confounding effect.

Our response:
The differences between each constitutional type and corresponding counter type (TE vs. NTE, SE vs. NSE, and SY vs. NSY) are now presented in the Results section on page 11 (additionally in last sentence of page 12). Almost all physical and biochemical characteristics were significantly different. Therefore, the constitution-associated effects of the FTO variant were re-analyzed after adjusting for BMI (page 12).

We also explained the role of the BMI as a confounder in the association between FTO variants and SC types in the second paragraph of the Discussion section in the revised manuscript, on the basis of the relationship between FTO and increased risk for obesity and diabetes (page 14).

- Minor Essential Revisions

Comment 3: In Materials and Methods, the ethnicity of the subjects should be stated clearly.

Our response:
We have stated the ethnicity of the recruited subjects in the end of the Subjects part of Materials and Methods: “The KoGES and KCMS study participants were all of Korean ethnicity.”

Comment 4: In order to minimize the subjectivity for classification, the SCAT was used to classify subjects. The authors should explain what advantage SCAT has and how it can demonstrate the subjectivity.

Our response:
In the revised manuscript, we have more clearly explained the advantage of SCAT: it provides common rules for diagnosis among SC medicine practitioners who have various points of view. This is added to the last paragraph of the Background section (pages 5 and 6) as follows: “Recently, a diagnosis method for SC typing has been developed by integrating the holistic diagnostic processes of SC medicine practitioners and quantitative data such as face, body shape, voice, and questionnaire information including personality traits [23]. The SC analysis tool (SCAT) is suggested to be a diagnostic model representing common rules among practitioners who have various points of view [23].”

Comment 5: The authors should state the criteria for inclusion and exclusion of participants in Materials and Methods.

Our response:
We have stated the criteria for inclusion and exclusion of participants in Subjects section of Materials and Methods section of the revised manuscript as follows: for KoGES, “The criteria for inclusion of subjects in the study were availability of SCAT and genome-wide genotype data. The criteria for exclusion were as follows: a history of cancer; classification as a TY type, which is extremely uncommon in Korea (0.03–0.1%) [1]; low-quality genome-wide genotype data as previously described [25] including gender inconsistencies, cryptic relatedness, and problems with genotype call rate and sample contamination.” and for KCMS, “The 2,519 individuals (904 men and 1,615 women) for the replication analysis were recruited from 22 Oriental medical clinics for the Korea Constitution Multicenter Study (KCMS) from 2006 to 2012, after applying the above-mentioned inclusion and exclusion criteria except that for low-quality genotype data.”

In addressing the inclusion and exclusion criteria, we are sorry that we found errors on the number of subjects in both KoGES and KCMS populations: the number of SC subgroups (SC types and corresponding counter types) in KoGES was all 1,905; the total number of KCMS subjects was 2,519. According to the changes in the number of SC subgroups in KoGES, the association results were changed a little, although there were no alterations in association trends. Therefore, we have revised the number errors throughout the revised manuscript, largely in Subjects section of Materials and Methods section and in Tables.

**Comment 6:** There are continuous variables and categorical variables in Table 1. The footnotes should be revised.

**Our response:**
We have rewritten the footnote of Table 1 as “Values are presented as mean (standard deviation) or as %.”

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**Reviewer 3 (Dr. Tran Quang Binh)**

**- Major Compulsory Revisions**

**Comment 1:** No data support for association of each of “Cautious”, “low-appetite”, and “slim body” with the obesity-risk variant of FTO. Thus, title of paper is not appropriate to the content of findings. It should be modified.

**Our response:**
The title was changed to “The obesity-risk variant of FTO is inversely related with the So-Eum constitutional type: Genome-wide association and replication analyses.”

**Comment 2:** It is better if SC type is used as a single multinominal variable (TE, SE, SY, and TY) instead of 3 binary variables.

**Our response:**
Thank you for your advice. However, unfortunately, there were overlaps between the SC types as mentioned in our response to the first comment of reviewer 1, so we
could not classify the subjects exclusively into TE, SE, and SY types. Therefore, we used 3 binary variables in the GWAS. In the revised manuscript, we explained this in Statistical analysis section of the Methods section (page 9) as follows: “Not all subjects could be exclusively assigned to one specific SC type based on the SCAT data, especially in the case of the SE and SY types (Additional Figure 1). Therefore, the association between the SC type and the genetic variants was assessed using each binary SC variables (TE vs. NTE, SE vs. NSE, and SY vs. NSY), instead of using a single multinomial variable (TE, SE, and SY). Here, the NTE, NSE, and NSY were corresponding counter types of TE, SE, and SY, respectively. To compare characteristics of the study subjects between SC types and corresponding counter types, Mann-Whitney U test and chi-square test were used for continuous and categorical variables, respectively.”

- Minor Essential Revisions:
  Comment 3: In the results, the author should present only their finding in the present study. Several sentences need to move to the discussion part or methods:
  
  Comment 3.1: Page 10: “There were no problematic samples with low call rates (<96%), cryptic relatedness, gender inconsistencies etc., in KoGES individuals in this study, as described in the previous GWA analysis [25].”
  
  Our response:
  The indicated sentence was removed, because a similar sentence was already described in first paragraph of Subjects section of Materials and Methods section (pages 6).
  
  Comment 3.2: Page 10: “Interestingly, the variants for determining the SE type were located in intron 1 of FTO, as shown in a regional plot constructed using LocusZoom [30]”----using LocusZoom should be presented in methods.
  
  Our response:
  The phrase in the Results section, “using LocusZoom for a genomic region of 800 kb centered on the peak SNP,” was moved to the second paragraph of the Statistical analysis section of the Materials and Methods section as a complete sentence: “A regional association plot for a genomic region of 800 kb centered on the peak SNP was constructed using LocusZoom [27].” (page 10)
  
  Comment 3.3: Page 11: “…those (for example, rs9939609 and rs8050136) have been well known to be involved in obesity-related traits [31, 32].”
  
  Our response:
  The indicated sentence was moved and rewrote to second sentence of second paragraph of the Discussion section (page 14) in the revised manuscript as follows: “… since the rs7193144 is strongly linked with rs9939609 and rs8050136 variants in the Asian HapMap population which variants are well known to contribute to obesity and diabetes through affecting BMI [32-34]..”
**Comment 3.4:** Page 11: “Since the FTO variants are associated with obesity-related traits [31, 32].”

**Our response:**
The indicated phrase was removed from the results, as the content was similar to that of the above-mentioned sentence, which was moved to the Discussion section (please refer to our response to comment 3.3). The original sentence was rewritten as: “The effects of the FTO variant rs7193144 on the SC type were reanalyzed after adjusting for BMI…”

**Comment 4:** Page 14----“replication analysis in 5,478” is not correct in conclusion.

**Our response:**
We have removed the number 5,478 from the expression.

**Comment 5:** Page 15: “The lower predisposition to weight gain and metabolic disorders, together with cautious personality, reduced appetite, and slim body, are characteristic of the SE type.” is not finding of the study. Thus, it cannot be written in conclusion.

**Our response:**
The sentence was removed from the Conclusions section.

- **Discretionary Revisions:  
  **Comment 6:** Add number of subject in Tables

**Our response:**
As the number of subjects were already presented in Tables 1 and 2, we added the number of subjects only in Table 3.

**Comment 7:** Title of Table 3 needs to include “after adjusting age, sex, and BMI”.

**Our response:**
In the revised manuscript, we have included “after adjusting for age, sex, and BMI” in the title of Table 3.