Author's response to reviews

Title: Genetic variations in APPL2 are associated with overweight and obesity in a Chinese population with normal glucose tolerance

Authors:

Shan Jiang (joyce830909@yahoo.cn)
Qichen Fang (fqichen@yahoo.com.cn)
Weihui Yu (shadow1201@163.com)
Rong Zhang (rongzhang11@hotmail.com)
Cheng Hu (alfredhc@sjtu.edu.cn)
Kun Dong (kundong2009@yahoo.cn)
Yuqian Bao (byq522@163.com)
Chen Wang (wangchenchen06@gmail.com)
Kunsan Xiang (sphxiang@163.com)
Weiping Jia (wpjia@sjtu.edu.cn)

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Author's response to reviews: see over
Dear Editor and Reviewers,

Thank you very much for your careful reading and important suggestions, which really helped us improve our study and this manuscript. We modified the manuscript according to your suggestion.

We really hope that you will now find our manuscript to be acceptable for publication in *BMC genetics*.

Thank you again for your helps and efforts in improving this manuscript.

Best regards,

Sincerely,

Weiping Jia, M.D., Ph.D.
Director, Professor of Medicine
Department of Endocrinology and Metabolism, Shanghai Jiao Tong University Affiliated Sixth People’s Hospital

Reviewers' comments:

Reviewer: Francis Vasseur
Reviewer's report:
The manuscript entitled Genetic variations in APPL2 are associated with overweight and obesity in a Chinese population with normal glucose tolerance by Shan Jiang et al. has been greatly amended and thus greatly improved.
Moreover many additional investigations requested have been performed.
All the initial remarks from #1 to #7; #12 to #17, #19 and #20 have been taken into account.
Some previous remarks still need clarifications.
Minor essential revisions
#a: In the table 1 the authors should avoid the SE for « age » and prefer to present the standard deviation SD.
Response: Actually, in the table 1 we present the mean±SD for « age ». I am sorry for this typo in the legend of the table 1. We modified the legend of the table 1 in the revised manuscript. We wrote, "Data are shown as mean ± SD or median (interquartile range)." Thank you very much.

#b: In the legend of table 4 the authors wrote « Log transformed values were used for p, Beta and SE values ». Do they mean that the obesity related phenotype under investigation was log transformed before use in the GLM? If so it would be clarified in the legend of the table 4.
Response: Yes, we meant that the obesity related phenotypes under investigation were log transformed before used in General Linear Regression Model. So we wrote in the revised legend of the table 4 and additional file 2 (table S1), "Obesity related measures under investigation were log transformed before used in General Linear Regression Model." Thank you.
Following the previous remark and the authors’ responses, some questions and remarks must still be drawn. Regarding the GLM analysis of the whole set of subjects investigating the putative link between rs2272495 and obesity-related phenotypes, it is surprising that when stratifying according to the 3 groups: «normal » « overweight » and « obese », none analysis was significant. Indeed, when focusing on the most significant phenotype (BMI) as reported in the present version Table 4 (with a 0.008 p value), the median BMI values are almost equal across the 3 genotypes in the normal weighted population (21.702; 21.713; 21.770 for the TT; CT; CC), likewise in the overweight population (25.637; 25.467; 25.646). Thus with such « equal » median values across the genotypes and the quite unsignificant p values (0.413; 0.497) that do not reflect even a trend toward association, at least in these two subgroups of patients an association between BMI and rs2272495 that would not reach significance because of a lack of power when stratifying is questionable. A similar conclusion may be drawn for the obese group as mean BMI values are similar across genotypes and p value is unambiguously not significant. Indeed inside each group, there is no evidence suggesting increasing BMI according to genotypes and each strata appears homogeneous according to the obesity-related phenotype under study even if this phenotype differs between strata. Thus it appears that the associations with obesity-related phenotypes and rs2272495 are only the reflection of the association between rs2272495 and overweight, and between rs2272495 and obesity as it is reported in Table 4. According to the data presented in the manuscript, it is difficult to conclude to an association with obesity-related phenotypes but only of an association with overweight and obesity. Does the distribution of obesity-related phenotypes under study even if this phenotype differs between strata. Thus, it appears that the associations with obesity-related phenotypes and rs2272495 are only the reflection of the association between rs2272495 and overweight, and between rs2272495 and obesity as it is reported in Table 4. According to these results, we agree that we cannot draw solid conclusions that rs2272495 were associated with obesity-related measures in our subjects. The associations between rs2272495 and obesity-related phenotypes may only reflect the association between rs2272495 and overweight/obesity. We had a discussion on this point in the revised manuscript. We wrote, "Secondly, regarding the table 4, rs2272495 were associated with obesity-related measures. However, when stratifying according to the 3 subgroups (normal weighted, overweight and obese), no significant association was observed in subgroups. According to these results, we agree that we cannot draw solid conclusions that rs2272495 were associated with obesity-related measures in our subjects. The associations between rs2272495 and obesity-related phenotypes may only reflect the association between rs2272495 and overweight/obesity. We had a discussion on this point in the revised manuscript. We wrote, "Secondly, regarding the table 4, rs2272495 were associated with obesity-related measures. However, when stratifying according to the 3 subgroups (normal weighted, overweight and obese), no significant association was observed in subgroups. According to these results, we agree that we cannot draw solid conclusions that rs2272495 were associated with obesity-related measures in our subjects. The associations between rs2272495 and obesity-related phenotypes may only
reflect the association between rs2272495 and overweight/obesity.

#d: answering to #11 the authors think that the GLM remains more suitable for their data. There is no reason to reject the GLM procedure but it was only suggested to use this GLM procedure in a non-parametric context using the Conover and Iman method. Unfortunately although the authors claim they see increasing values of (i.e.) BMI according to the number of C alleles most of the Dunnet contrast tests they report were unsignificant. Thus it is impossible to conclude to an additive model and the hypothesis of a recessive model as pointed out by my previous estimated calculation remains a possibility that the authors should have tested. However referring to #c the results do not allow to conclude to an association with obesity related phenotypes and thus the manuscript should be amended accordingly.

Response: We added analyses of the associations between rs2272495 and obesity-related phenotypes using a recessive model in the revised table 4. The associations between rs2272495 and obesity-related measures were significant both under an additive model and a recessive model. Therefore, in the revised results section, we wrote, "And the associations between rs2272495 and obesity-related measures were still significant under a recessive model (Table 4)."

Take all results (Table 4 and see Additional file 5) together, according to the review’s suggestion, we had a discussion in the revised manuscript (referring to #c).

#c: Regarding the answer to #18 although the multivariable linear regression model the authors used was performed under an additive model, a significant result does not implies that biologically it is an additive model according to the presence of a given allele. Moreover as association strongly suggests a recessive model (see #11 of the previous report) and as the Dunnet contrast tests they report were unsignificant and do not allow the authors to conclude to a given genetic model, the affirmation by the authors of an undemonstrated additive (co-dominant) model remains an over interpretation of the data.

Response: We added analysis using a recessive model in the revised manuscript. The associations between rs2272495 and obesity-related measures were significant both an additive model and a recessive model. However, Dunnet contrast tests we report were unsignificant (see #11 response of the previous report). And when stratifying according to the 3 subgroups (normal weighted, overweight and obese), no significant association was observed in subgroups. Take all results together, we cannot draw solid conclusions that rs2272495 were associated with obesity-related measures in our subjects. Therefore, we had a discussion on this point in the revised manuscript (referring to #c). Thank you very much.

Some new suggestions arise from the new amended version of the manuscript.

Major revision

#f: The sentence at line 153 « carriers of a greater number of rs2272495 C allele exhibited higher WHR » that refers to table 4 is an over interpretation of the data as median WHR is 0.849 for TT and 0.849 for CT, thus WHR is not higher in patients having one C allele as compared with those having none.

Response: Thank you. We revised this sentence in the revised manuscript. We wrote, "moreover, rs2272495 was also associated with waist circumference (beta=0.006, \( p=0.002 \)), hip circumference (beta=0.003, \( p=0.024 \)) as well as WHR (beta=0.003, \( p=0.017 \)) after adjusting for
age and sex under an addictive model (Table 4). "

Minor revisions
#g: As the authors now pooled overweight and obese patients, as they did at line 144 it would be more convenient to present all the results as: “…evidence of associations with overweight/obesity…” rather than “…evidence of associations with overweight and obesity…”.
Response: We used “…associations with overweight/obesity…” instead of “…associations with overweight and obesity…” to present all the results throughout the revised manuscript. Thank you.

#h: at line 200 the authors should use « underlying » and at line 206 « Bonferroni ».
Response: All these mistakes were modified in the revised manuscript. Thank you.

#i: at line 208 the sentence may be misunderstood: one may think that the authors tempted a replication that was negative. They should write that they had not the opportunity to perform a replication another independent sample.
Response: We amended this sentence in the revised manuscript. We wrote, "Thirdly, although we found associations of rs2272495 and rs1107756 with overweight/obesity and obesity-related measurements, we had not the opportunity to perform a replication another independent sample."
Thank you.
Reviewer: Nabila Bouatia-naji
Reviewer's report:
The revised version of the manuscript is much improved.
Minor comment:
Please change the title line 103 to "In silico prediction of the functionality of APPL2 SNPs". The current title is misleading and suggests biological assessment.
Response: Thank you very much for your kind comments. We have changed the line 103 to "In silico prediction of the functionality of APPL2 SNPs" in the revised manuscript.