Author’s response to reviews

Title: Estrogen Receptor 1 Gene Polymorphisms Are Associated with Metabolic Syndrome in Postmenopausal Women in China

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Title: Estrogen Receptor 1 and 2 Gene Polymorphisms associated with the metabolic syndrome in Postmenopausal Chinese Women

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Response to Reviewers' comments

Dear Dr. Pradeepa,
We thank you for your careful consideration of our manuscript. We appreciate your response and overall positive initial feedback, and made modifications to improve the manuscript. After carefully reviewing the comments made by the Reviewers, we have modified the manuscript to improve the presentation of our results and their discussion, therefore providing a more complete context for the research that may be of interest to your readers.

We hope that you will find the revised paper suitable for publication, and we look forward to contributing to your journal. Please do not hesitate to contact us with other questions or concerns regarding the manuscript.

Best regards,

Reviewer #1

1. One of the major concerns is that correction for multiple testing has not been done. Conclusions should be drawn depending on whether the findings are statistically significant after correction.

   Response: We thank the reviewer for the comment. We corrected the analyses, and obtained approximately the same results.

2. The other major concern is whether the study has adequate power to arrive at a meaningful conclusion. Power calculation should be presented in the manuscript.

   Response: We agree with the Reviewer. BMI was used to calculate the power, as in a previous study [1]. The mean difference of BMI between the two groups was 25.6-24.4=1.2, that is, the allowable error of BMI between the two groups was 1.2. The total standard deviation σ was 2.9. The formula of power calculation is: (Sorry Reviewer #3, there is a statistical formula...
that can't be uploaded, if you need , I can send it to you by email.) Hence, power was calculated as $1-\beta=94.5$.

3. Was the genotyping checked by direct sequencing. This is very important as a quality control measure. Atleast 10% samples should be checked by sequencing.

   Response: We agree with the Reviewer. Ten percent of the samples had been checked by direct sequencing. The service of direct sequencing and BLAST were provided by Sangon Biotech (Shanghai) Co., Ltd. (Supplementary Figures S1-S3).

Minor suggestions:

4. The size of fragments after restriction digestion to be mentioned in the methodology.

   Response: We agree with the Reviewer. The sizes were added to the manuscript.

5. Comparison of biochemical characteristics genotypewise has been done on all study individuals. The study participants must also be classified into cases and controls and the biochemical characteristics can be compared genotypewise.

   Response: We agree with the Reviewer. We added new results, as below.

   Regarding XbaI genotypes, in the control group, there were significant differences in FBG, TG, total cholesterol, HDL-C, and LDL-C among the three genotypes (all $P<0.05$). In the MetS group, there were differences in BMI, TG, total cholesterol, and LDL-C (Supplementary Table S1). Regarding RsaI (B5) genotypes, in the control group, there were significant differences in fasting insulin and HOMA-IR among the three genotypes (all $P<0.05$). In the MetS group, there were differences in LDL-C and HOMA-IR (Supplementary Table S1). Regarding RsaI (B5) genotypes, in the control group, there were significant differences in fasting insulin and HOMA-IR among the three genotypes (all $P<0.05$). In the MetS group, there were differences in LDL-C and HOMA-IR (Supplementary Table S3). Regarding AluI (B8)
genotypes, in the control group, there were significant differences in BMI and total cholesterol among the three genotypes (all $P<0.05$). In the MetS group, there were differences in age and TG (Supplementary Table S4). Regarding PvuII genotypes, in the control group, there were significant differences in estradiol among the three genotypes (all $P<0.05$). In the MetS group, there were differences in age, BMI, systolic BP, HDL-C, and HOMA-IR (Supplementary Table S5).

6. The results of LD estimation between the SNPs ($D'/r^2$ values) should also be presented.

Response: We agree with the Reviewer. The results were added as Supplementary Table S1.

7. The title of the manuscript can be modified as only ESR1 gene polymorphisms have shown association and not the ESR2.

Response: We agree with the Reviewer. It was edited accordingly.

Reviewer #2

1. The title of the article says "Post-menopausal Chinese women". But the study was done in one medical center. The title is misleading because one medical center does not represent Chinese women. The title should be modified accordingly

Response: We agree with the Reviewer. It was edited accordingly.

2. The methods section should include more information about the methods of estimation of each of the biochemical and clinical parameters. How were blood pressure and waist circumference measured? Was LDL-C measured or calculated using Friedewald formula?
Response: We thank the Reviewer for the comment. We added the requested information to the manuscript.

3. How was the sample size of the study determined? Did the authors make a sample size calculation? If so, a short description about the sample size calculation should be included in the methods section.

Response: We agree with the Reviewer. BMI was used to calculate the power, as in a previous study [1]. The mean difference of BMI between the two groups was 25.6-24.4=1.2, that is, the allowable error of BMI between the two groups was 1.2. The total standard deviation σ was 2.9. The formula of power calculation is: (Sorry Reviewer #2, there is a statistical formula that can't be uploaded, if you need, I can send it to you by email.) Hence, power was calculated as 1-β=94.5.

4. Abstract section: Results, line 3: higher than those in the controls.

Response: We thank the Reviewer for the comment. It was corrected.

5. Which internationally accepted guideline was used in the diagnosis of metabolic syndrome? A cut point of ≥85 cm is used for waist circumference. Is it a Chinese specific guideline? Also, what is the criteria used for general obesity? These details should be clearly mentioned in the methods section.

Response: All cases were diagnosed according to the Chinese type 2 diabetes Prevention Guide (2013) diagnostic guidelines [2]. MetS was diagnosed by the presence of three or more of the following factors: waist circumference ≥85 cm; fasting blood glucose (FBG) ≥6.1 mmol/L or glucose of 2 hours post glucose–load ≥7.8 mmol/L or known diabetes; serum TG ≥1.70 mmol/L; HDL-C <1.04 mmol/L; and blood pressure ≥130/85 mmHg or treated hypertension.
6. RsaI1082A>G and AluI1730A>G polymorphisms are within the ESR2. In Table 2, it is mentioned that these polymorphisms are in ESR1. This should be corrected.

Response: We are sorry. This is a typo. It was corrected.

7. It is mentioned that "The minor alleles of the XbaI and PvuII gene polymorphisms in the homozygous or heterozygous form were associated with higher waist circumference, fasting serum insulin, HOMA-IR, and NAFLD (%)." This association is not demonstrated in the results section. Only descriptive data has been included.

Response: We thank the Reviewer for the comment. Regarding PvuII genotypes, in the control group, there were significant differences in estradiol among the three genotypes (all P<0.05). In the MetS group, there were differences in age, BMI, systolic BP, HDL-C, and HOMA-IR (Supplementary Table S5).

8. It is also mentioned that "Among subjects with the Rsa I polymorphism, carriers of the minor G allele in the homozygous or heterozygous form (AG or GG genotypes) had higher TG, LDL-C, fasting serum insulin, and HOMA-IR. In subjects with the Alu I polymorphism, carriers of the minor G allele in the homozygous or heterozygous form (AG or GG genotypes) had higher total cholesterol and LDL-C". This data is also not shown.

Response: We thank the Reviewer for the comment. Regarding RsaI (B5) genotypes, in the control group, there were significant differences in fasting insulin and HOMA-IR among the three genotypes (all P<0.05). In the MetS group, there were differences in LDL-C and HOMA-IR (Supplementary Table S3). Regarding AluI (B8) genotypes, in the control group, there were significant differences in BMI and total cholesterol among the three genotypes (all P<0.05). In the MetS group, there were differences in age and TG (Supplementary Table S4).
Reviewer #3

1. The study groups comprised of 150 subjects with metabolic syndrome (cases) and 154 controls. The study appears to be underpowered due to the sample size, although the authors have mentioned this as the limitation of the study. Authors need to provide the power calculation for the study to substantiate the findings.

   Response: We thank the Reviewer for the comment. BMI was used to calculate the power, as in a previous study [1]. The mean difference of BMI between the two groups was 25.6-24.4=1.2, that is, the allowable error of BMI between the two groups was 1.2. The total standard deviation $\sigma$ was 2.9. The formula of power calculation is: (Sorry Reviewer #3, there is a statistical formula that can't be uploaded, if you need, I can send it to you by email.) Hence, power was calculated as $1-\beta=94.5$.

2. Correction for multiple testing using Bonferroni should also be mentioned.

   Response: We agree with the Reviewer. It is now mentioned.

3. Although the study subjects were matched for age, the BMI of the cases included in the study is significantly higher than the controls. Therefore the Odds ratio has to be adjusted for BMI to establish the risk better, given that BMI is an independent risk factor for the development of metabolic syndrome. Odds ratio should also be adjusted for the other confounding factors related with the disease.

   Response: We agree with the Reviewer. We now provide the new Table 5 with the multivariable adjustment.

4. The genotype counts of the AluI SNP of ESR2 gene, as given in the Table 2, does not add up to the total number of Control group (i.e. n=154) included in the study. Moreover their genotype and allelic frequencies do not match with the given genotype count.
Response: We thank the Reviewer for the comment. We verified the data and they checked correctly.

Minor Comments:

In Table 2: The genotype and allele distribution of RsaI and AluI SNPs are given under the subheading ESR1 gene. It is ESR2 and not ESR1.

Response: We thank the Reviewer for the comment. It was corrected.

References
