Author’s response to reviews

**Title:** Association analysis between the tag single nucleotide polymorphisms of DENND1A and the risk of polycystic ovary syndrome in Chinese Han women

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**Version:** 2  **Date:** 28 Oct 2019

**Author’s response to reviews:**

Dear Editor,

Re: MGTC-D-19-00211R1 (Research Article) Zhu et al "Association analysis between the tag single nucleotide polymorphisms of DENND1A and the risk of polycystic ovary syndrome in Chinese Han women".

We would like to thank the editor and the reviewers for their constructive suggestions and comments. We hereby resubmit our revised manuscript to BMC Medical Genetics.

Editor and Reviewers’ major suggestions and comments are responded point-by-point as follows. Q stands for “question”, and A stands for “answer”. We hope that the manuscript can now be accepted for publication in this journal. We are looking forward to your favorable decision.

To Editor:
Q1: The information of the sample should be appropriately addressed, both clinical information as well as population information, as pointed out by the reviewers, respectively.
A1: We agree with the editor and reviewers and we have added such information in the revised manuscript (page 7).

The PCOS patients and non-PCOS controls were of the Han ethnicity, recruited from Drum Tower Hospital in Nanjing and Department of Obstetrics and Gynecology, Anhui Medical University in Hefei, Eastern China.

Patients with PCOS were diagnosed according to the 2003 Rotterdam Criteria (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). The Rotterdam Criteria requires at least two of the following indicators for diagnosis of PCOS:

1. Oligo- and/or anovulation
2. Clinical and/or biochemical signs of hyperandrogenism
3. Polycystic ovaries
   and exclusion of other aetiologies (congenital adrenal hyperplasias, androgen-secreting tumours, Cushing's syndrome).

The women in the control group came to see a doctor for other reasons (such as tubal factor infertility or their husbands' infertility). Some of them have given birth to one child or more. Their menstrual cycles are normal (< 32 days) and exclusion criteria were hirsutism, insulin resistance, other property of hyperandrogenism and obesity.

Q2: The tagSNP selection should be justified, in particular, the rsID# should be counter-checked and verified.

A2: We have counter-checked and verified the tagSNP selection. The details were showed in the answer for question 3 of reviewer 2 and in the manuscript (Page 6.8).

Reviewer 1:

Q1: It would be nice to see more information about the subject recruitment including the distribution of PCOS subtype? Were they recruited from clinics? How the authors chose controls? If all controls were taken serum androgen measurements, what was the main reason for biochemical tests in table 1?

A1: Due to the small number of samples after subtype classification, which is not described in the article, no further subtypes were considered. This is a drawback in this research. We will continue to investigate the effect of subtypes on SNP in a future project.

The PCOS patients and non-PCOS controls were of the Han ethnicity, recruited from Drum Tower Hospital in Nanjing and Department of Obstetrics and Gynecology, Anhui Medical University in Hefei, Eastern China.

The women in the control group came to see a doctor for other reasons (such as tubal factor infertility or their husbands' infertility). Some of them have given birth to one child or more. Their menstrual cycles are normal (< 32 days) and exclusion criteria were hirsutism, insulin resistance, other property of hyperandrogenism and obesity.
Q2: What cut-offs did the authors use to define hyperandrogenism (both clinical and biochemical)? Have these assays and cut-offs been established and validated in the population?

A2: We used the cut-offs described below to define hyperandrogenism (both clinical and biochemical). These assays and cut-offs have been established and validated in Chinese population. These contents can be found in the diagnosis standard of PCOS for Chinese women established in 2011 and revised in 2018, and Chinese clinicians have reached a consensus.

Clinical hyperandrogenism:
The clinical indicator of androgen excess is the presence of hirsutism, acne and the androgenic alopecia. The improved ferriman-gallwey (mf-g) scoring system was used to evaluate hyperpigmentation. It is suggested that a mf-g score ≥5 should be used to diagnose hyperpigmentation. The Global Acne Grading System (GAGS) can be used for grading acne severity. Androgenetic alopecia was scored with Ludwig visual.

Biochemical hyperandrogenism:
During the follicular phase of the normal menstrual cycle, average serum testosterone concentrations are around 0.43 ng/ml and peak at 0.68ng/ml, with levels greater than 0.7 ng/mL (2.44 nmol/L), known as hyperandrogenemia. The diagnosis is based on increased blood testosterone >2.44 nmol/L. There is no positive correlation between total T and the degree of clinical symptoms. In order to identify the origin of the lesion from ovary or adrenal cortex, ACTH stimulation test can be used.

Q3: Why AMH and metabolic parameters are not measure in the subject population?

A3: Indeed, it makes sense to do the test for AMH. However, due to the conditions and the cost of the experiment, we did not conduct the corresponding test.

Reviewer 2:

Q1: The genetic structure of the samples should be evaluated prior to any association test, as Han Chinese is not a homogenous population. The location of samples should be clarified.

A1: This comment has also been raised by Editor and Reviewer 1 and we have added such information in the revised manuscript (page 7).

Q2: The sample size used in this study is not large enough, and may lead to limited power of the association test. I understand the difficulties in sample collection, but this issue should be discussed.

A2: We agree with this reviewer and appreciate his or her understanding. We will continue collecting more samples and expect to have a larger sample size in future studies. We have commented this issue in the conclusions in the revised manuscript (page15-16).

Q3: It is not clear how the 5 tag SNPs were selected. "we systematically studied SNP polymorphisms in the promoter region of DENND1A ...", "... one SNP was selected in each region of the DENND1A gene ..." -- which region exactly? These descriptions are confusing. Also, I suggest they present a full picture of the LD pattern on all the genotyped loci (not just for the 5 SNPs as shown in Figure 1), and reason why these 5 SNPs were chosen as tags in subsequent analyses. They intended to give the details in the second section of Methods "Charactetization of LD and selection of tag SNPs", but the whole paragraph is about genotyping experiments, and does not match the subtitle. I suggest they rename this section, and set up a new paragraph for the tag SNP determination.

A3: We agree with the reviewer and have presented the reason why these 5 SNPs were chosen as tags in subsequent analyses in the paper. Based on the NCBI database, a full picture of the LD pattern on all the genotyped loci of DENND1A has been presented as a supplementary data (Figure S1-1, S1-2). In this picture, all the SNPs of
DENND1A are shown including those sites (such as rs2479106 and rs10818854) mentioned in the references. As most studies suggest that the polymorphism site rs2479106 in the DENND1A gene is related to PCOS susceptibility, we chose the other four SNPs which have strong relation with rs2479106 in Block 12. The pair-wise correlations between rs2479106 and these SNPs are the same (with the same value 95 in the diamond) (Fig. 1, S1). Finally, five tagging SNPs including rs2479106, rs2768819, rs2670139, rs2536951 and rs2479102 were selected for the following association study.

For the title of the second section of Methods, we agree with the reviewer and we have already renamed this section as “Analysis of polymorphism genotypes”.

Q4: As mentioned in Backgrounds, there are several susceptible loci for PCOS reported in previous study. I think it is necessary to show how these priori candidate loci relate to the 5 tag SNPs and how they correlate with PCOS in these data here. It is not only a validation, but also show us the similarity and differentiation of populations to the disease risk.

A4: Unfortunately, we did not study the relationship between these sites and the five sites we chose. It is true that many loci are associated with the onset of PCOS. However, as these sites are not the main topics of this article, we did not present more details in the background introduction in this work.

Other minor issues/suggestions:
Q1: Since all the samples studied are Han Chinese, the title can be more precise, for instance, "Association analysis between the tag single nucleotide polymorphisms of DENND1A and the risk of polycystic ovary syndrome in Han Chinese women"
A1: We agree with the reviewer and have modified the title accordingly.

Q2: Line 2, Page 9: MAF < 0.05 should be MAF > 0.05?
A2: Yes, MAF < 0.05 should be MAF > 0.05. We have corrected this error in the revised manuscript.

Q3: Line 5-6, Page 9: the illustration of Figure 1 (b for case and c for control) is quite different from the figure legend (b for D' and c for R²). I don't understand this figure.
A3: Indeed this is an error. We have revised the legend in the manuscript (Page 10).

Q4: One of the 5 SNPs, rs2479102, was misspelled as rs24791902 in quite a lot of sentences in the manuscript. rs24791902 is not an available ID in dbSNP. Also, which version of SNP/gene annotation was used in this study (GRCh37 or GRCh38? Ensembl or Refseq?).
A4: We thank the reviewer for pointing out this error. We have corrected it.
In this study, NCBI36, Ensembl release 54 was used.

Yours sincerely,
On behalf of my coauthors
Yong Wang Ph.D.