Author's response to reviews

Title: Association between CYP19 gene SNP rs2414096 Polymorphism and polycystic ovary syndrome in Chinese women

Authors:

Jia-Li Jin (jjllily@126.com)
Jing Sun (sijnju@163.com)
Yun-xia Cao (caoyunxia6@126.com)
Xiao&\#8211;Ke Wu (xiaokewu@nju.edu.cn)
Feng-jing Liang (gqg149@sina.com)
Hai-xiang Sun (stevensun@sohu.com)
Lu Ke (kkb9832@163.com)
Long Yi (yilong@nju.edu.cn)
Yong Wang (yongwang@nju.edu.cn)

Version: 3 Date: 1 November 2009

Author's response to reviews: see over
Dear Dr. Scott:

Ref.: MS: 1512193024296179

Association between CYP19 gene SNP rs2414096 Polymorphism and polycystic ovary syndrome in Chinese women

Thank you very much for your email of 25 Sep 2009 with which you sent me the reviewers’ comments and editorial suggestions on my manuscript. I would also like to thank the reviewers for their work on our manuscript.

According to the reviewers’ suggestions and comments, we have revised the manuscript. I hope this version of the manuscript will encourage your acceptance for publication.

The questions rose by the reviewers are responded as follows:

Reviewer Agathocles Tsatsoulis:

Thank you for your comments.

The revision according to Major Compulsory Revisions:

1. In the control group, the concentration of T was lower than that in the women with PCOS, even if the activity of the aromatase has decreased, the conversion efficiency of androgen also can be constant by increasing the quantities of the aromatase, so the changes may not be obvious. The patients with PCOS generally have higher concentrations of T and the
aromatase is basically at saturation, so the activity level of the aromatase will directly correlate to the conversion efficiency of androgen, making it easier to be detected with statistical significance in the patients. While the E2 / T ratio was not statistically significant among the various genotypes in the control group, the ratio in the AA genotype was still higher than that in the other two genotypes.

2. The fact that our results were different from that of Petry et al’s may lie in these points.

1) The selection criteria for the subjects is different. Petry et al chose the girls with PP (Age at assessment = 9.8-10.9) as participants, while our subjects were women with PCOS (Age at assessment = 21-33). Adolescent girls are subject to physical and psychological changes dramatically, and their endocrine levels fluctuate more to internal and external environmental factors.

2) The frequency distribution of alleles A/G is different between Asians and Europeans.

3) In Petry et al’s experiment, the SNP 50 genotypes from girls with PP were not in Hardy–Weinberg equilibrium (P < 0.05). Their sample size is small and it may not be representative of the overall ensemble, thus affecting the statistical accuracy.

3. The fluctuations of E2 levels in the follicular phase are relatively large, and the normal reference range of laboratory diagnosis is
91.75-275.25 pmol / L. Thus the SD value of the experimental data is relatively large. In addition, the required minimum sample size is 139 to reach statistical significance by sample size estimation. The number of our patients was 386 and that of the control group was 298, both far exceeding the required minimum sample volume, so we thought there is no need to further increase the number of subjects.

The revision according to Minor Essential Revisions:

1. We have rephrased our discussion in the MS according to your suggestion.

2. We have added how the information about the age at menarche was obtained in the Materials and Methods section.

3. We have revised the discussion.

4. We’ve tried to revise the language used in the text and asked a native English speaker to help checking our paper for grammar and syntax.

Reviewer DJ Marioli:

Thank you for your comments.

According to your suggestions and comments, we have revised our MS and we would answer your questions as follows.

The revision according to Major Compulsory Revisions:

1. We have added a separate table with the general characteristics of PCOS patients (anthropometric and hormonal measurements) expressed as
mean value± SD in order to better define the phenotypic characteristics of PCOS patients as a whole (Table 1). We have added the table 1 into our MS(Table 1).

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Age* (years)</th>
<th>AAM* (years)</th>
<th>BMI* (kg/m²)</th>
<th>FSH (IU/L)</th>
<th>LH* (IU/L)</th>
<th>LH/FSH</th>
<th>T* (nMol/L)</th>
<th>E2* (pMol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCOS</td>
<td>386</td>
<td>26.6 ± 4.2</td>
<td>14.4 ± 1.6</td>
<td>22.7 ± 3.5</td>
<td>7.3</td>
<td>15.1 ± 6.9</td>
<td>2.4</td>
<td>2.9 ± 1.2</td>
<td>223.6 ± 136.2</td>
</tr>
<tr>
<td>CONT</td>
<td>298</td>
<td>31.5 ± 2.4</td>
<td>14.4 ± 1.3</td>
<td>21.4 ± 2.3</td>
<td>7.1</td>
<td>4.5 ± 1.1</td>
<td>0.6</td>
<td>1.1 ± 0.7</td>
<td>166.1 ± 142.5</td>
</tr>
</tbody>
</table>

Note: *P < 0.05 vs control.

According to your suggestion, we have further divided the patients' group into three subsets (table 2). In every subset, the concentrations of T among the three genotypes had no statistical differences. However, we didn't discuss this result in the manuscript.

<table>
<thead>
<tr>
<th></th>
<th>AA</th>
<th>AG</th>
<th>GG</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.4</td>
<td>0.738±0.4692</td>
<td>0.92±0.3218</td>
<td>1.15±0.2828</td>
<td>0.362</td>
</tr>
<tr>
<td>1.4-2.1</td>
<td>1.693±0.1281</td>
<td>1.706±0.2184</td>
<td>1.759±0.22</td>
<td>0.6819</td>
</tr>
<tr>
<td>&gt;2.1</td>
<td>3.61±1.3018</td>
<td>3.652±1.2082</td>
<td>3.796±1.2464</td>
<td>0.7868</td>
</tr>
</tbody>
</table>

2. For the participants who have a spontaneous bleeding episode, the peripheral blood was obtained during the third to the fifth day of the menstrual cycle. And for those who had amenorrhea (menstrual cycles > 6 months), the peripheral blood was obtained at any time. Perhaps some samples were collected in the ovulatory phase. So in the ulterior investigations, we would strictly design the experimental considerations to
improve the experiment.

3. The results of our experiment were different from that of Petry et al. The possible explanations may be as follows:

   1) The selection criteria for the subjects is different. Petry et al chose the girls with PP (Age at assessment = 9.8-10.9) as participants, while our subjects were women with PCOS (Age at assessment = 21-33). Adolescent girls are in a physical and psychological period when dramatic changes are taking place, and their endocrine is more influenced by internal and external environmental factors.

   2) The frequency distribution of the alleles A/G is different between Asians and Europeans.

   3) In Petry et al’s experiment, the SNP 50 genotypes from girls with PP were not in Hardy–Weinberg equilibrium (P < 0.005). Their sample size is small which may not be representative of the ensemble, thus affecting the statistical accuracy.

   It's a pity that we didn't measure the concentrations of estrone and androstenedione.

4. We didn't observe any differences in T levels among genotypic subgroups of PCOS patients. The possible explanations may be as follows:

   1) Aromatase is the key enzyme for the conversion of androgen to estrogen, and its activity can affect the efficiency of transformation. However it cannot affect the synthesis of androgen. A variety of factors
can affect the synthesis course of androgen. Although there is no significant statistical difference, the level of testosterone in AA genotype was lower than that in the other two genotypes.

2) In the group of women with PCOS, there was no difference of the testosterone levels among the three genotypes. But in the AA genotype, the E2 / T ratio was higher than the other two genotypes, indicating the androgen conversion in AA genotype is more efficient when the level of androgen is high (aromatase is in the saturated status). It may also indicate that higher aromatase activity exists in AA genotype.

5. We have revised the conclusion.

6. We have rephrased the reference section in the MS according to the BMC medical genetics guidelines.

The revision according to Minor Essential Revisions:

1-12 and 14-19. We have corrected the MS according to your suggestions.

13. We designed new primer pairs for this study, which is not related to any of the previous studies in the literature.

20. We have clarified that the values in Table II are expressed as mean value±SD.

To editor's suggestions:

According to the comments and suggestions of reviewers and editors,
we have revised our manuscript. I hope this version of the manuscript will encourage your acceptance for publication.

If you have any questions, please don’t hesitate to contact me.

Sincerely yours,

Yong WANG, PhD.

Jiangsu Key Laboratory of Molecular Medicine & Center for Public Health Research, Medical School of Nanjing University, Nanjing 210093, China

Email: yongwang@nju.edu.cn