Author’s response to reviews

Title: Association between gene polymorphisms of voltage-dependent Ca2+ channels and hypertension in the Dai people of China: a case-control study

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Author’s response to reviews:

Dear editor Dr.Gunadi

We sincerely thank you and all reviewers for the valuable feedback. We feel lucky that our manuscript went to these reviewers as the comments from them not only help us with the improvement of our manuscript, but suggested some neat ideas for future studies. Please do forward our heartfelt thanks to those experts.

All the changes were marked in red text. In addition, we also have a native English speakers double-checked the English for the revised version. We hope the new manuscript will meet your standard. Below you will find our point-by-point responses to the reviewers’ comments:

Response to comments on Association between gene polymorphisms of voltage-dependent Ca2+ channels and hypertension in the Dai people of China: a case-control study

Reviewer #1 (Remarks to the Dr. Maharani)
1. Regarding the inclusion criteria of subjects: Why the maximum age for subjects was not set? The older the subject, the more environmental effect will affect the blood pressure, and it become less likely to be caused by genetic factors.
Response: Thank you for your question. Indeed, as subject getting older, the influence of the environment becomes greater. Setting a maximum age limit does help to highlight genetic factors and reduce environmental interference. However, as most of the Dai people live in the underdeveloped rural areas, their cognizance of hypertension is extremely poor. The most of the patient did not realize that they had hypertension before we collected the information of subject, which make it difficult to determine the specific age of onset. So, we didn’t set a maximum limit.

2. What the authors can conclude regarding the finding that the significant associations were found in both dominant and additive model?
Response: Special thanks to you for your good comments. We have made a conclude in discussion section:
We found one positive SNP (rs10425859), which suggest an increased risk for the people carried the A minor allele on this SNP. (OR Additive = 1.269 CI 95% Additive = 1.030-1.563), in CACNA1A in the Dai population after logistic regression analyses.
In Dai people, another positive SNP (rs2365293), which suggest an increased risk for the people carried the T minor allele on this SNP (ORDominant = 1.335 CI 95% Dominant = 1.002-1.778).

We found three other positive SNPs (rs17539088, G minor, ORDominant = 0.732 CI95% Dominant = 0.554-0.966, ORAdditive = 0.769 CI95%Additive = 0.616-0.960; rs16917217, G minor, ORDominant = 0.737 CI95% Dominant = 0.558-0.973, ORAdditive = 0.776 CI95% Additive = 0.622-0.969; and rs61839222, A minor, ORDominant = 0.737 CI95% Dominant = 0.558-0.973, ORAdditive = 0.776 CI95% Additive = 0.622-0.969), which suggest a decreased risk, in CACNB2 after logistic regression analyses.

In fact, it makes sense that the significant associations were found in both dominant and additive model, because the additive model can be seen as a dominant model with a dose effect. In the dominant model, People with the AA or Aa genotype would get disease. In the additive model, People with the AA or Aa genotype also got sick, but the blood pressures of people with AA are higher than the people with Aa.

3. In the Discussion section, I think it would be better if the authors can add more explanation about the location of each polymorphisms in the gene, which domain, and what could be the consequences for each significant polymorphism.
Response: Thank for this good suggestion, we added the explanation such as: "The positive SNPs showed in this study all locate on the intron. These SNPs may be linked to susceptible mutations, which make them show positive results in association analysis. The frequency of susceptible mutations may be very low, so it is difficult to obtain significant positive results by association analysis. However, during evolution, especially in these ethnic groups with a relatively narrow genetic background, the mutations are likely to be linked to Tag SNPs, so that they can be detected in our study. These findings may help us understand the genetic causes of hypertension from a unique perspective." (line 276-283, page 9-10)

4. For Methods section, please mention the total concentration of each PCR mix (DNA, primers and dNTPs) instead of its volume.
Response: Thanks. We have corrected these mistakes based on your suggestions:
First round reaction mix for PCR contained 3.2 µl of ddH2O, 1 µl of 10X buffer, 2 µl of 50nM primer, 0.8 µl of 2.5mM dNTP, 0.1 µl of 5U/ul Taq polymerase, 2 µl of DNA, 1 µl of 100mM Mg2+. (line 140-142, page 5)
The second round PCR mix included 3.6 µl of ddH2O, 2 µl of 10X buffer, 3.6 µl of 2uM barcode, 0.8 µl of 2.5mM dNTP, 0.1 µl of 5U/ul Taq polymerase, 10 µl of DNA, and 1 µl of 100mM Mg2+. (line 145-147, page 5)

5. The amount of subjects included in the abstract shouldn't it be 1034 instead of 1221?
Response: "1221" is the amount of samples we initially obtained. However, in the statistical analysis, we filtered out the part at the critical values (±5mmHg). We apologize for the misunderstanding and have made changes accordingly: A total of 1034 samples from Dai individuals were collected, of which 495 were used as cases, and 539 were used as controls. (line 38, page 2)

6. Methods and Results section: "gender" should be changed into "sex".
Response: We are very sorry for our incorrect writing. We have changed all the “gender” to “sex”.

7. Please have the manuscript checked for its grammatical errors.
Response: We have invited a native English speaker to check the grammar in the hope of reducing grammatical errors.

Reviewer #2 (Remarks to the Dr. Yunus)
1. Abstract:
- Shorter background is better.
- Detail explanation related to BP measurement in method section should be added.
Response:
① Thanks very much for your suggestion. We have made some changes in the background and made sure that the original meaning has not changed. It was also listed below. (line 32-36, page 2): "Abnormal calcium homeostasis related to the development of hypertension. As the key regulator of intracellular calcium concentration, voltage-gated calcium channels (VDCCs), the variations in these genes may have important effects on the development of hypertension. Here we evaluate VDCCs variability with respect to hypertension in the Dai ethnic group of China."
② We also added BP measurement in method section. (line 34-41, page 2):
  Blood pressure was measured using a standard mercury measurement method, three times with a rest for 5 min, and the average was used for analyses.

2. Introduction:
- In the last paragraph, it is better to describe in more detail about the background of Dai people. Why is it important to be chosen as subjects. Description about the study (number of subject, data measurement, statistical analysis) should be stated in Methods section, not in Introduction.
Response: Thanks for your very thoughtful suggestion. We have changed the last paragraph of introduction section. It was also listed below. (line 86-96, page 3-4): "Dai people natively live in Yunnan of China for centuries. As they have special feelings for their land, one of their characteristics is to firmly hold their native land. Most of them live in the
river valleys and flat areas surrounded by mountains. Therefore, the geographical barriers make it extremely difficult for them to communicate with groups beyond the boundary of Dai ethnic. Meanwhile, due to the closure of the society, the lack of understanding and even the estrangement with other ethnic groups, Dai people adopted the custom of intermarriage within their own group and rarely intermarried with other ethnic groups before the founding of New China. As a result, compared with other ethnic groups (such as Han), Dai people have relatively small genetic differences and pathogenic spectrum among individuals."

Descriptions about the study were moved to the Methods section.

3. Results & Discussion:
- There is no explanation related to OR, is there any increased risk or decreased risk, since this study is a case control study.
- Comparison with other ethnicities should be emphasized in more detail.
- Study limitations should be mentioned, i.e., related to sample size, further prospective studies, etc.
Response: Thank you for your suggestion. We've added the corresponding content:

① We found one positive SNP (rs10425859), which suggest an increased risk for the people carried the Aminor allele on this SNP. (ORAdditive = 1.269 CI95%Additive = 1.030-1.563), in CACNA1A in the Dai population after logistic regression analyses
In Dai people, another positive SNP (rs2365293), which suggest an increased risk for the people carried the Tminor allele on this SNP (ORDominant = 1.335 CI95%Dominant= 1.002-1.778). (line 239-241, page 8-9)
We found three other positive SNPs (rs17539088, Gminor, ORDominant = 0.732 CI95%Dominant= 0.554-0.966, ORAdditive = 0.769 CI95%Additive = 0.616-0.960; rs16917217, Gminor, ORDominant = 0.737 CI95%Dominant= 0.558-0.973, ORAdditive = 0.776 CI95%Additive = 0.622-0.969; and rs61839222, Aminor, ORDominant = 0.737 CI95%Dominant= 0.558-0.973, ORAdditive = 0.776 CI95%Additive = 0.622-0.969), which suggest a decreased risk, in CACNB2 after logistic regression analyses. (line 251-257, page 9)

② We added some comparison with other ethnicities such as: "Compared with the Han people who have a complex genetic heterogeneity, the Dai have a genetical homogeneity and smaller pool of susceptibility genes. The disease gene spectrum of hypertension may be narrow in this population. A few disease gene mutations responded for the hypertension in Dai people, which provides an advantage in finding susceptibility variations of hypertension in this special group. (line 270-275, page 10)"

③ There are some limitations in the current study. First, as a cross-sectional study, we were not able to determine the causal relationship between VDCCs and hypertension. Second, since all the participants were Dai people, the generalisability of the results may not extend to non-Dai populations. Although the main constraint of this study was the small sample size of the Dai group, the statistical power is enough for the number of analysed SNPs. Further functional studies and association analyses in larger samples and other populations should be conducted to confirm our results. (line 280-290, page 10)
According to the reviewer’s comments, we have revised the manuscript extensively. If there are any other modifications we could make, we would like very much to modify them and we really appreciate your help. We hope that our manuscript could be considered for publication in your journal. Once again, thank you very much for your comments and suggestions.

Yours sincerely,
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