Author's response to reviews

Title: The Neck-Region Polymorphism of DC-SIGNR in Peri-Centenarian from Han Chinese Population

Authors:

Hui Li (lihui19801019@gmail.com)
Cheng-Ye Wang (tkchYWang@yahoo.com.cn)
Jia-Xin Wang (wang174344@163.com)
Nelson Leung-Sang Tang (nelsontang@cuhk.edu.hk)
Liang Xie (xeronxie@hotmail.com)
Yuan-Ying Gong (yuanyinggong@yahoo.com)
Qing-Peng Kong (kongqp@yahoo.com.cn)
Ya-Ping Zhang (zhangyp1@263.net.cn)

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

Thank you very much for giving us the opportunity to revise our manuscript. We have performed additional analysis in reply to reviewers’ comments, and to further substantiate our conclusion. Furthermore, the manuscript has been modified considerably, with text revised and the structures of some paragraphs (discussion section) adjusted. Meanwhile, a native speaker has been invited to help us edit the language. The changes to the text have been highlighted in red color.

Here is a list of our responses to reviewers’ comments.

**Reviewer: 1**

The authors need to described in more details the recruitment procedures and the clinical/demographic characteristics of the study participants. The subjects were randomly selected from how many subjects, from where (hospitals, clinics, home, etc). What was the method used to randomly assigned the subjects. What is the % of peri-centenarian individuals in Dujiangyan community. What is the distribution of the different variables associated with life span (smoking, incomes, chronic diseases, etc) among the cases and controls.

**Answer:** We agree with the comments made by the reviewer. Although the ratio of peri-centenarian people in Dujiangyan is high (about 0.18% in 2004), when compared to the general population, it is still rare. So it is not easy to collect samples “randomly”. Given that these long-lived individuals were unrelated and their living places dispersed, we met the peri-centenarian individuals at their homes, accompanied by 2 professional doctors. On the condition that the peri-centenarian individual is healthy, we collected as many participants as possible to ensure the population size. Totally we collected more than 500 peri-centenarian individuals (age≥94 for female and age≥90 for male), then we randomly selected about equal numbers of male and female: 181 males and 180 females. We say “randomly” here just mean that there is no other standard when selecting samples except for ensuring the longevity age. The demographic characteristics of the study participants were described in our previous work as mentioned in our text. All the 324 matched controls collected from Dujiangyan in Sichuan province were confirmed to be healthy subjects by normal physical examination. Unfortunately, variables as smoking, incomes, chronic diseases...
and etc. were not available in this study. Now we removed the “randomly” to avoid any confusion and made corresponding revision in our text.

**Reviewer: 2**

*Major compulsory revisions:* The study design needs to be reconsidered. The prevalence of the various polymorphic alleles and genotypes of DCSIGNR must be surveyed and studied in a random general population of Hans Chinese. Based on the frequency distribution of the genotypes and alleles obtained in the general population, the sample size should be decided in consultation with a statistician. The distribution of the VNTR polymorphism in the general population should then be compared to that in the eri-centenarians to determine if any particular allele or genotype is associated with longevity.

*Other comments:* "The authors should consult a statistician for sample size calculation."

**Answer:** We agree with the comments. A new section describing the sample size determination is now added to the “Methods” section after consulting a statistician. The sample size required to detect an effect caused by various alleles were calculated based on their respective allele frequencies in Han (as suggested by the reviewer).

**Reviewer: 3**

*There are a few things that I believe the authors should clarify/correct before the manuscript is ready for publication.*

1- In the Results and Discussion section it is not clear to me what do the authors mean by linkage disequilibrium between the 6/7 genotype and other functional loci. How can a specific genotype in this region be in linkage disequilibrium with another functional variant? What could happen is that the alleles 6 or 7 (or eventually both) could be linked with a functional variant what in that case should be revealed by an association between these alleles and longevity; not the genotype? I think there explanation of LD should be clarified.

**Answer:** We agree with the comments made by the reviewer. We amended the text and it now reads “The result indicated that there was more 6/7 genotype in female longevity group than that in control group (Table 2) with a marginal level of significance (5.56 vs. 1.28%, p=0.041), but this difference was no longer significant after correction by Bonferroni method. It seems that the 6/7 genotype may show a false positive association due to multiple testing. However, we cannot exclude a
functional role of 6/7 genotype and the hypothesis that the effect of DC-SIGNR on longevity maybe different between males and females. Considering the relative small sample size in this study, our hypothesis needs to be replicated with larger sample size.” Furthermore, the effect of the genotype 6/7 on longevity, if any, should not be due to LD.

2- In addition, I think their discussion should not be driven by the a priori hypothesis that there must be an association between the neck-region of DC-SIGNR and longevity. In page 10 the authors give multiple explanations why they couldn’t find an association without ever mentioning the most likely scenario; that there is actually no association between DC-SIGNR neck-region variation and longevity. In this sense, I believe that some parts of the discussion should be re-phrased (mainly pag 10).

Answer: we re-phrased sentences in page 10 of Discussion section as the reviewer suggested and we also commented in the text that DC-SIGN might not have an effect on longevity: “After all, it was equally likely that DC-SIGNR gene did not have a role in healthy aging of human beings.”

We hope that our revised manuscript meets your requirements.

Sincerely yours,

Ya-Ping Zhang