Author's response to reviews

Title: Interactions between Social/ behavioral Factors and ADRB2 Genotypes May be Associated with Health at Advanced Ages in China

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Author's response to reviews: see over
Author’s response to reviews

Title: Interactions between Social/behavioral Factors and ADRB2 Genotypes May be Associated with Health at Advanced Ages in China

1. Report on how we respond to the Editor’s comments in carefully revising our paper

This is a very good study and the paper is in a good shape. However, there are some minor or moderate issues needing the authors to address. In addition to the reviewers’ comments, I have following additional comments to share with the authors. Please address all comments raised by the reviewers one by one and highlight the changes in the revised paper.

Your comment: Exercise and leisure activity likely refer to same thing, i.e., mobility or activity. Maybe consider combining them together if possible.

Authors: We carefully considered whether it is good to combine them, but we are finally convinced that it is better to let them stand as two variables. This is because Social-leisure activities score is based on frequency of participation in seven activities: gardening, raising domestic poultry/pets, playing cards or mah-jongg, participating in organized social activities, reading newspaper/books, watching TV and/or listening to the radio, and personal outdoor activities. Respondents who report engaging in the activity once or more per week are coded 1; otherwise, coded as 0. We then sum the seven scores (range from 0 to 7) and dichotomize the social-leisure activities score as $\geq 2$ vs. $<2$. Participation in the social-leisure activities depends on senior adults’ interests and it is also subject to time limit. Thus, it is not necessarily the case that the larger number of social-leisure activities an elder participate in, the more active he/she is, and it may not make sense to use a continuous variable to measure the social-leisure activities. After trying different combinations, we found that dichotomization of the social-leisure activities score as $\geq 2$ vs. $<2$ is the best choice.

If we combined social-leisure activities with regular exercise, it would imply that the role of regular exercise in the combined index was equal to one of the seven social-leisure activities, but this may not be appropriate, as regular exercise is substantially more important in enhancing health than any of the seven social-leisure activities.

Your comment: Last sentences in conclusions in Abstract: I would expect that it is very difficult, at least till today, for public health program to consider (even to know) individual's genetic profiles in implementation. At this stage, the implication of the findings is mainly academic.

Authors: Following your suggestion, we revised the wording “health promotion programs” into “near-future health promotion programs” in that sentence. Such implication is likely possible as this field is developing very quickly.

Your comment: Is there any gender difference? I would expect some interesting gender differential or interactions.

Authors: Sample size of the oldest-old to whom PKU lab did the genotyping with very limited pilot grant support is small especially for males, and thus we are not able to do analysis for males and females separately, but we did control for gender in all of our multivariate statistical analysis. We also tried the logistic regression analysis for males and females separately and the results are basically consistent with our presented two-genders combined

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models; the statistical significance levels were reduced substantially due to sample size reduction. We added an endnote to explain this.

Your comment: Pages 9-10: some justification for inclusion of demographic variables and family supports/connections would be useful.

Authors: As you suggested, we have added justification indicating that these demographic and family supports/connections variables are associated with the dependent variables of health outcome being investigated, based on the literature and our understanding of the Chinese social context.

Your comment: Some negative emotion questions in the CLHLS are not necessarily stressors. For example, the perception or feeling of more uselessness when one gets older is just an attitude or a way to viewing one's life or tackling with one's daily events. It is an acceptance of natural change, which may not be bad in some cases. Similarly, regular exercise and social/leisure activities are not necessarily stress releasers. Furthermore, citations are need if the authors want to define stressors and stressor releasers.

Authors: We agree with you. Following your comments, we have now deleted the wording of “stressors” and “stress releasers” in our analysis. Instead, we used much more appropriate wording “negative emotion (related to stress)” and “regular exercise and social/leisure activities” (related to stress releasing).

Your comment: Page 12, Step 2: ANOVA is for continuous variables. I did not see where the authors used ANOVA. If it was not used, please drop it. And please be more specific to the current study when the authors describe the three-step procedure.

Authors: Following you comment, we carefully revised the text of the three-steps and they are more specific to the current study now by adding the variables we used with “e.g. or such as” in the parenthesis. We also replaced the wording of “We use” by “One uses” to describe the general three-step procedure which may be applied to other similar and relevant studies as well.

Your comment: The term "relative excess risk" may not be appropriate and may be confusing. First, only ORs, not Relative Risk (RRs), are produced by logistic regressions. Second, if OR=2.26, does that mean the excessive OR is 2.26? No, the excess OR is only 1.26 (2.26-1). Therefore, to avoid confusion, please phrase the text accurately.

Authors: The wording of “relative excess risk” to represent the odds ratio of the GxE interaction term was previously adopted from the reference 61, and we agree with you that it is not a good phase and it may cause confusion. Therefore, we have now replaced it by “odds ratio of the interaction term (ORIT)” in the footnote which has moved to be the note (3) of Tables 6a, 6b and 7.

Your comment: Several issues deserve attentions and corrections in Endnote 8. First, the authors noted that statistical software usually does not provide OR CI for an interaction term. This is true. However, the authors presented p values for interaction terms in both Tables 2 and 5. How did the authors calculate p value for interactions? Honestly, there is a very way to get it from any software, by just generating a new variable that includes all possible categories of two dummy interaction variables. If one is a continuous variable, the authors may either keep its all value or re-categorize it into an ordered variable with some limited and equal intervals. For example, if A and B are both dummy, then the new variable C has four categories: (1) A=0, B=0; (2) A=1, B=0; (3) A=0, B=1; and (4) A=1, B=1.
Authors: The note (3) of Tables 6a, 6b and 7 may clarify this issue: Because the $ ORIT = \frac{OR_{11}}{(OR_{10} \cdot OR_{01})}$ (ORIT is the odds ratio of GxE interactions, estimated and provided by the standard statistical software, in the regressions with two dummy variables and one interaction term) [47,61], $OR_{11} = OR_{10} \cdot OR_{01}$, ORIT. However, we do not know the statistically significant level (i.e., p value) of the $OR_{11}$ estimated based on $OR_{10}$, $OR_{01}$ and ORIT normally produced by the statistical software. Thus, we alternatively estimated $OR_{10}$, $OR_{01}$ and $OR_{11}$ and their p values by setting up three exclusive dummy variables $V_{10}$, $V_{01}$ and $V_{11}$ (without interaction term) in the regression equation: $V_{10} = 1$ if $E=1$ and $G=0$; $V_{01} = 1$ if $E=0$ and $G=1$; and $V_{11} = 1$ if $E=1$ and $G=1$, while considering $V_{00}$ ($E=0$ and $G=0$) as the reference group. In such alternative regression, the $OR_{11}$, $OR_{10}$, $OR_{01}$ and their significant levels are estimated and the ORIT can be calculated based on estimates of the $OR_{10}$, $OR_{01}$ and $OR_{11}$. Note that the $OR_{10}$, $OR_{01}$, $OR_{11}$ and ORIT estimated in such an alternative regression are exactly the same as those estimated in the normal procedure with two dummy variables and one interaction term.

Your comment: manuscript should be proofread. Below I list few that need revising.
(1) 1st sentence in results in Abstract: "... associated with good cognitive function;...."

Authors: corrected.

(2) Page 10: 2nd paragraph: Pay attention to balance quotations.

Authors: corrected.

(3) Page 11: top line: no " " after "three items."

Authors: corrected.

(4) Table 1: "MMSE good, MMSE moderate, and MMSE poor" need rewording. MMSE is a test or a scale (with a certain score range), which itself cannot be good, moderate or poor.

Authors: corrected.

(5) Table 1: the range for social/leisure activities score should be listed in the table.

Authors: corrected.

(6) Table 2: The authors need to tell readers that the left panel is based on ordered logistic regression, while the right panel is based on binary logistic regression.

Authors: corrected.

(7) relative excessive risk is not a correct term here.

Authors: “The relative excessive risk due to GxE interaction” is replaced as “GxE interaction terms”.

(8) Tables 3a and 3b should be dropped as one reviewer noted.

Authors: We moved a couple of sentences in the end of previous Section 3.2 and added several new sentences to formulate the new first paragraph of Section 3.3 to explain why the structural equation analysis is useful and necessary in this case as follows: As shown in Tables 3a and 3b, the Chi-squared tests show that the rGE correlation between carrying ADRB2 minor alleles and negative emotion cannot be ruled out (the estimates are
marginally significant, p <0.1). Moreover, we found that carrying the rs1042718 or rs1042719 minor allele is significantly and negatively associated with negative emotion (p <0.05), controlling for the socio-demographic characteristics of age, gender, rural/urban residence, education, family/social connections (marital status, proximity to children, and social/leisure activities score) and health practice (regular exercise) (see Table 4). Thus, the statistically significant GxE interactive terms between the ADRB2 genotypes and negative emotion presented in models I-A4 and I-B4 in table 2 may be confounded by the rGE correlation between carrying ADRB2 minor alleles and negative emotion, and the ADRB2 genotypes may have an indirect association with cognitive function through negative emotion (see Figure 1 and its associated discussions in Section 2.3).

(9) The title of Table 4 is not very clear. Also, logistic regression is ordered or binary?

Authors: Title of Table 4 is revised and “binary logistic regression” is indicated now.

(10) Table 6a: I am unable to figure out where 2.27, 1.19 and 2.87 are from. Also, pay attention to text "Model II-A2" and "Model III-A2." I could not find "Model III-A2."

Authors: The estimates in Table 2 were updated shortly before submission of our manuscript, but we forgot to update Table 6a accordingly. We now updated the numbers and moved the previous endnote 8 to be the note (3) of Tables 6a, 6b and 7, in order to enable readers to easily understand how the estimates were produced. The model code is corrected now.

(11) Table 6b: a full table note is needed. Do not use "same as in Table X" or the like. Also, I did not find Model III-B2 and Model III-B3 in Table 2. I am also unable to find any number in this table from Table 2.

Authors: The estimates in Table 2 were updated shortly before submission of our manuscript, but we forgot to update Table 6b accordingly. We now updated the numbers and the full notes are added.

(12) Table 7: a full table note needs to be completed. I did not find "0.55" nor "0.65" in Table 5. The table title should be revised to indicate what kind of ORs. That is, what is (are) dependent variable (s): MMSE score or SRH?

Authors: The added note (3) explains how "0.55" nor "0.65" which are not in table 5 were estimated. We now indicated that the dependent variable of table 7 is MMSE score.

2. Report on how we respond to Referee One’s comments in carefully revising our paper

Re: ‘Interactions between Social/ behavioral Factors and ADRB2 Genotypes May be Associated with Health at Advanced Ages in China’

BMC Geriatrics

Research article

General comment:

Reviewer’s comment: Would it be possible that the carrier of rs1042718 or rs1042719 alleles also carries APOE4 allele or other genotype such as FOXO1A-209? If the authors can rule out this gene-gene interaction in the “Results” section or address this question in the “Discussion” section, it will not only improve the quality of the current paper as a whole, but
also highlight an important question of the gene-gene interaction that has been intriguing longevity and health related researchers.

Authors: We only genotyped FOXO and ADRB2 genes (not including APOE4) for these Mainland Chinese samples using our very limited pilot grant from Peking University. As you suggested, we conducted 20 additional regressions to explore whether GxG interactions between rs1042718 or rs1042719 and each of the 2 SNPs of FOXO1A and 3 SNPs of FOXO3A are significantly affecting cognition and SRH. It turns out that only three estimates of the odds ratios of the GxG interactions \([\text{rs1042719} \times \text{FOXO3A292}]\); \([\text{rs1042718} \times \text{FOXO3A936}]\); \([\text{rs1042719} \times \text{FOXO3A936}]\) are significant \((p<0.05)\), while all of the other 17 estimates are not significant.

Moreover, the focus of this article is to explore the effects of interactions between social/behavioral factors and ADRB2 genotypes rather than the effects of GxG interactions, as indicated by the title of this article. Thus, we did not include the GxG estimates in this article, while we have added it as one of the future research perspectives in the conclusion section.

Specific comments:

Reviewer's comment: 1. Background, 2nd paragraph: the authors quoted the paper Zhao et al (2012) “based on genotype data from 893 long-lived Han Chinese aged 90+ at baseline, most of whom survived to age 100+, and 893 middle-age controls, our group’s recent population association study identified two synonymous single-nucleotide polymorphisms (SNPs) of rs1042718 (C/A) and rs1042719 (G/C) that are significantly associated with longevity, namely, survivorship from middle-age to advances age \((P=0.001-0.0001, \text{adjusted for gender})\)”. Based on the paper, page 1095 in the “Results” section, the authors wrote “The Han Chinese population in this study consisted of 963 long-lived”. Thus, can the authors clarify this number?

Author's: In fact, Zhao et al. (2012) basically used genotype data from 893 long-lived Han Chinese CLHLS participants' blood dry-spot samples collected in 1998 at baseline of CLHLS. At the final stage of revisions and resubmission of Zhao et al (2012), the group led by Prof. Tian added 70 long-lived individuals' full blood samples collected by Prof. Tian's lab from the hospitals' normal health examinations (rather than CLHLS), mostly for some biological functional analysis to strengthen the explanations of the results. Thus, the final total number of long-lived samples was 963, as stated in Zhao et al. (2012). However, the lately-added 70 long-lived cases were NOT CLHLS participants and the phenotype data needed for the GxE analysis are not available for them, and thus we did not include them in this paper, which caused confusion. We have added endnote 3 to explain this to avoid any confusion. Thanks for your thoughtful comment to point this out.

Reviewer's comment: 2.2 Measurements, independent variables, and behavioral and social participation factors, 2nd paragraph: the authors used the 3 questions asked in the CLHLS to operationalize “negative emotion” which is considered as stressor and they reported that 45.3% of oldest-old answered “yes” to one of the 3 questions, i.e. do you often feel the older you get, the more useless you are? It would be more appropriated, if the authors can explain how they define “Yes”, as there are 5 possible answers, namely, always, often, sometimes, seldom, never and not able to answer. A clarification is also needed for another 2 questions of “negative emotion”.

Authors: Following your suggestions, we have added the following sentences in the text: “Interviewees who answered "always" or "often" for these questions were regarded as "yes"; those who answered "sometimes" "seldom" or "never" were regarded as "no"; and those who were unable to answer the question were excluded as missing cases.
Reviewer’s comment: On the other hand, the reviewer is intrigued by another personality question that appeared in the CLHLS questionnaire “B2.1 Do you always look on the bright side of things?” Will this variable play a partial role as “positive emotion” or “counter-stressor” to increase the odds ratio of good SRH and have association of carrying the ADRB2 minor alleles with cognitive function? How about other depressive symptoms such as using GDS, will this variable generate a confounding effect on the “negative emotion”?

Authors: We tried to do the regression using “looking on the bright side of things” as an independent variable. We found that it is significantly associated with MME score and SRH, but its interaction with the ADRB2 genotypes are not significantly associated with MMSE score and SRH at all. Thus, we do not include it in our article. In the baseline of CLHLS, we did not collect other depressive symptoms and we could not construct the general depression symptoms (GDS).

Reviewer’s comment: 3.3 Results, path analysis employing the structural equations method, 1st paragraph: the authors wrote “we conducted path analysis employing ….. with MMSE, indirect association with MMSE through its correlation (rGE) with negative emotion, and the association between ADRB2 genotype-negative emotion interactions and MMSE. These results correspond to Table 5, but in Table 5, first column “Model”, “Direct association with “Negative emotion” and “indirect association of ADRB2 with MMSE” was not in line with in text. There is a typo in Table 5 “association” instead of “6ssociation”.

Authors: the wording in Table 5 and the related text are revised to correspond each other. The typo is corrected.

Reviewer’s comment: Discussion, 3rd paragraph: The authors wrote “For example, both SNPs of rs1042718 and rs1042719 are highly correlated and in strong linkage disequilibrium (70%).” This sentence should come from the paper Zhao et al. 2012 of which it refers to men in two geographically isolated populations. The authors may overlook the in-text citation.

Authors: Yes, you are right, and the citation of Zhao et al. (2012) is now added. Thanks!

3. Report on how we respond to Referee Two’s comments in carefully revising our paper

Reviewer’s report:
This is nicely written paper showing that synonymous coding polymorphisms in the ADRB2 gene may be associated with health outcomes and interact with social factors in older Chinese population.

Minor comments:

Reviewer’s comment:
1) Introduction, page 5, last paragraph. The statement that the effect of genes at advanced ages becomes more pronounced is not entirely correct. Many other studies (e.g., papers by Ewbank about APOE) show also that the effect of genes at advanced ages diminishes.

Authors: We agree with you that the statement “the effect of genes at advanced ages becomes more pronounced” is not entirely correct. We have revised that part of the text as follows and newly cited Ewbank (2002): “Note that some previous studies also indicated that, in general, genetic impacts on health and longevity are more profound at advanced ages [27], perhaps due to some unobserved and un-investigated heterogeneities including the effects of interactions between genetic and social/behavioural factors; but the other study found that the effects of APOE4 on mortality diminishes with age [28]. More importantly, the numbers of oldest-old have
been increasing much more dramatically than any younger age groups in many countries while the oldest-old much more likely need health and daily living care. These facts imply that focusing on the oldest-old is a useful way to investigate the effects of genetics and their interactions with social/behavioural factors on healthy aging. However, almost all previous studies in this field focused on young and middle aged adults and few had large numbers of oldest-old subjects.

Reviewer’s comment:
2) Sensitivity to cut-offs in definition of cognitive function should be tested because cutoffs in the MMSE are age and education sensitive. For example, people with fewer years of education (that is the case in this study) typically perform more poorly than individuals with higher education and, therefore, cutoff for them is smaller.

Authors: We have tested the sensitivity of cut-offs in definition of cognitive function, and current three categories cut-off is the best for this particular sample, and this is explained in endnote 5. We also tried different cut-off points for different education attainments, but the results remain almost the same, as the majority of this oldest-old sample had no or very low education level.

Reviewer’s comment:
3) It would be helpful you describe why you started with the dominant model rather than with the additive one.

Authors: We tried additive model, but the results are not ideal, because it double the complication for presenting 1 and 2 copies of carrying the minor allele, and moreover the category of 2 copies are most likely un-significant as its sub-sample size is often too small.

Reviewer’s comment:
4) Page 10, last line. It looks that parentheses are excessive there.

Authors: the parentheses are deleted now.

Reviewer’s comment:
5) To my opinion Section 3.3 does not add too much because Tables 3a and 3b show no substantial correlation between polymorphisms and negative emotions. Accordingly, the models for each outcome are pretty independent based on the results in Tables 3a and 3b. This is seen as no difference in the effects for interactions in Table 5 and 2.

Authors: We moved a couple of sentences in the end of previous Section 3.2 and added several new sentences to formulate the new first paragraph of Section 3.3 to explain why the structural equation analysis is useful and necessary in this case as follows:

As shown in Tables 3a and 3b, the Chi-squared tests show that the rGE correlation between carrying ADRB2 minor alleles and negative emotion cannot be ruled out (the estimates are marginally significant, p <0.1). Moreover, we found that carrying the rs1042718 or rs1042719 minor allele is significantly and negatively associated with negative emotion (p <0.05), controlling for the socio-demographic characteristics of age, gender, rural/urban residence, education, family/social connections (marital status, proximity to children, and social/leisure activities score) and health practice (regular exercise) (see Table 4). Thus, the statistically significant GxE interactive terms between the ADRB2 genotypes and negative emotion presented in models I-A4 and I-B4 in Table 2 may be confounded by the rGE correlation between carrying ADRB2 minor alleles and negative emotion, and the ADRB2 genotypes may have an indirect association with cognitive function through negative emotion (see Figure 1 and its associated discussions in Section 2.3).

6) Analysis in Section 3.4 is very useful.

7) Table 5: please correct “6association” as “association”.

Authors: corrected.
**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**
I declare that I have no competing interests