Author's response to reviews

Title: Serotonin transporter gene polymorphism (5-HTTLPR) L allele interacts with stress to increase anxiety symptoms in Chinese adolescents: a multiwave longitudinal study

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Author's response to reviews: see over
September 27, 2015
Mr. Carlo Rye Chua

Re: Manuscript 3132956017373473 entitled “Serotonin transporter gene polymorphism (5-HTTLPR) L allele interacts with stress to increase anxiety symptoms in Chinese adolescents: a multiwave longitudinal study”.

Dear Mr. Carlo Rye Chua,

Thank you for your letter of September 15, 2015 and we would like to thank you and the two reviewers for your carefulness and helpful comments. We have revised the manuscript carefully conforming to the journal style and responded to the reviewer’s comments point by point. The changes we have made are highlighted with yellow marker in the revised manuscript.

Response to Reviewer 1: Dr. Kelly Benke
Reviewer's report:
The authors have adequately addressed most of the previous concerns raised, however, the interpretation of their model does require further adjustment:
Major Compulsory Revisions
1. It is stated in the methods that the alleles are coded -1,0,1 (for LL, SL and SS respectively). Thus, a positive term for genotype (beta for 5HTT-LPR = 0.32) should mean that those with SS are highest under a low stress condition, but Figure 1 shows the opposite. The slopes appear to follow what intuitively a negative coefficient would mean, but if those with the LL genotype and high stress are truly at the highest anxiety level, would not the lines for SS and LL cross at some point? Either the figure is incorrect, or there is more detail here that needs to be explained, perhaps relating to the fact that ALEQ is centered around an individual’s mean, however, it will be confusing to the reader without some explanation.
Response: Thank you very much for your effort and suggestions on our manuscript. Because of a positive beta for 5-HTTLPR and a negative beta for 5-HTTLPR × stress interaction, the lines for SS and LL should cross at a point that the stress scores are very close to 0. In other words, SS carriers’ anxiety scores should be higher than LL carriers’ only when stress scores are very small. Here “low” or “high” defined by us means 1.5 within-subject standard deviation above or below individual’s mean level of stress. Low stress in Figure 1 is not close to 0, so SS carriers have lower anxiety scores at this point. We added more details about the figure as following, which have been highlighted with yellow on page 11, line 19-22, page 12, line 1 and page 25 line 4-7.

To present the form of this interaction, the model summarized in Table 4 was used to calculate predicted anxiety symptom scores for participants with SS, SL and LL genotypes who experienced a low or high level of stressful life events (plus or minus 1.5 × mean within-subject standard deviation) without controlling for age, gender or depressive symptoms (see Figure 1).

The high/low levels of stressful life events meant plus/minus 1.5 × mean within-subject standard deviation. Although increases in stressful life events were all associated with
increases in anxiety symptoms for individuals with different genotypes, LL carriers have a moderately heightened anxiety response to stress compared to SS carriers.

2. In Figure 1, the predicted curves are not explained in the methods or the figure legend. Does this represent girls or boys? At what CESD score, age, etc. is the prediction made? What is meant by low and high stress on the x axis? More detail is needed here for the reader.

Response: Figure 1 represents the whole sample because of no gender difference in 5-HTTLPR × stress interaction. CES-D score and age are covariates and are controlled for in the model to improve reliability of interaction effect. To present different predicted slopes between three genotype groups, we calculate six predicted anxiety scores for subjects with SS, SL and LL who experience a low or high level of life events (plus or minus 1.5 within-subject standard deviation) without controlling for depression scores and age. But if the prediction is made at definite values of CES-D score and age, we will miss some information. Because it means predictive effect of stress on anxiety when a individual with definite depressive symptoms and at a definite age. So, Figure 1 summarizes the relationship between stress and anxiety symptoms but does not represent all the HLM results. The means of low and high stress on the X axis has been added in results and figure legend on page 11, line 19-22, page 12, line 1 and page 25 line 4-7.

3. I’m not happy with the revision: “After controlling for age, gender, initial anxiety and depressive symptoms, a significant two-way, cross-level interaction between 5-HTTLPR and stress were detected (B=–0.08, p<0.01). Specifically, 5-HTTLPR acted as a moderating role in relationship between stress and anxiety symptoms. Compared with SS carriers, individuals with LL genotype exhibited higher levels of anxiety symptoms in relation to SLEs”.

The second sentence above is redundant – if it is interacting then it is a moderator. I would delete the second sentence and reword the third sentence. My suggestion (feel free to revise):

“After controlling for age, gender, initial anxiety and depressive symptoms, a significant two-way, cross-level interaction between 5-HTTLPR and stress were detected (B=–0.08, p<0.01). As stress levels increase, the anxiety level among SS carriers appears to increase at a slower rate compared to LL carriers. Another way of saying this is that LL carriers have a moderately heightened anxiety response to stress compared SS carriers.”

Response: Thank you for your suggestion. We revised the sentences as following, which have been marked in page 11, line 16-19.

After controlling for age, gender, initial anxiety and depressive symptoms, a significant two-way, cross-level interaction between 5-HTTLPR and stress were detected (B=–0.08, p<0.01). As stress levels increase, the anxiety level among SS carriers appears to increase at a slower rate compared to LL carriers.

Minor Revisions

1. The effect of 5HTT-LRP and stress is significant, but the effect size is modest. The authors should choose language reflecting this throughout the results and discussion, rather than stating the findings as significant. I feel the finding is a bit overstated as it now reads.
Response: To make the statements more moderate, we made some revisions as following, which have been marked on page 12, line 21-22 and page 13, line 1:

A two-way interaction of 5-HTTLPR and SLEs was detected in the current study. LL genotypes increase genetic vulnerability to the experience of anxiety symptoms slightly in response to daily stressors.

on page 15, line 12-14:

Additionally, the effect size of 5-HTTLPR × stress interaction was modest. It is important to replicate these results in future so that we can be more confident in this interaction.

on page 16, line 5-8:

Individuals with the L allele exhibited moderately heightened anxiety response to stress. If the results can be replicated, the current study will provide new evidence for exploring the roles of genetic and environmental factors in the pathological mechanism of anxiety.

Thank you again for your attention and consideration. We shall look forward to receiving good news from you.

Sincerely yours,

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