Author's response to reviews

Title: Interaction between a Serotonin Transporter Gene Promoter Region Polymorphism and Stress Predicts Depressive Symptoms in Chinese Adolescents: A Multi-wave Longitudinal Study

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Author's response to reviews: see over
May 2, 2013
Carlo Rye Chua, Ph.D.

Re: Manuscript 148373468928602 entitled “Interaction between a Serotonin Transporter Gene Promoter Region Polymorphism and Stress Predicts Depressive Symptoms in Chinese Adolescents: A Multi-wave Longitudinal Study”.

Dear Dr. Carlo Rye Chua,

Thank you for your letter of April 5, 2013 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 reviewers’ comments point by point. The revised manuscript was corrected thoroughly by a native English speaker, and the changes we have made are highlighted with yellow marker in the revised manuscript.

Response to Reviewer: Dr. Corina L Benjet
Reviewer's report:
This is a well-conducted study of the interaction between a serotonin transporter gene promoter region polymorphism and stressful life events on depressive symptoms in Chinese adolescents. This manuscript has several strengths. First and foremost of which is the prospective multi-wave longitudinal design. Secondly, the Chinese population provides evidence for a racial group for which there are limited data available for this particular hypothesis. The finding of a gender difference for this interaction is relevant; though I find it odd the lack of gender differences for depressive symptomatology in this age range as usually this difference emerges at puberty, but the authors note that lack of male-female differences in depressive symptomatology for this age group in the Chinese population has previously been reported. The analytic approach is appropriate and the discussion section contains a thoughtful discussion of the gender difference for this gene-environment interaction and of the limitations of the study. The manuscript is an important contribution to the field.

Response: Thank you very much for spending time and effort on this manuscript and for your high appraisal to our work. Our results shown that there was no male-female difference in depressive symptoms, which consisted with two former large sample studies in Chinese adolescents (eg. Tepper P, et al. Depressive symptoms in Chinese children and adolescents: parent, teacher, and self reports. J Affect Disord), but some abroad study demonstrated the differences (eg. Nolen-Hoeksema S, et al. The emergence of gender differences in depression during adolescence. Psychol Bull). The possible reason of this discrepancy should be studied in future research.

Response to Reviewer: Dr. Niki Antypa
Reviewer's report:
This is an interesting study examining a gene-environment interaction on depressive
symptoms of adolescent Chinese students. The longitudinal design of the study, which allowed for the examination of within subject variation, is a strong facet of the study. The article is well written and the analyses are appropriate. However, I have a few suggestions for improving and clarifying the manuscript:

Major compulsory revisions:

Results
1. The authors say that "There was an overall decrease in CES-D and ALEQ scores" - page 12. It would be important to examine whether the decrease in depressive symptoms is mediated by the decrease in ALEQ. The authors could examine this and briefly state the result.

Response: Thank you for your valuable suggestions. We have made some possible explanations for decrease in ALEQ and CES-D in discussion: "A possible reason for this result is that the students had just entered senior high school when the initial assessment was carried out. This major transition may have involved more competition and academic pressures, causing stress and depressive symptoms to have been at the highest levels. As the students acclimated to senior high school, their stress and depression levels may have decreased. In addition, there may have been a practice effect due to the repeated follow-up assessments." Since the main effect of stress on depressive symptoms which has been tested in HLM was significant, it was plausible that the decrease in ALEQ could lead to decrease in CES-D.

2. Figure: Error bars should be presented in the figures. Are depression scores in the y-axis collapsed (mean? Median?) across time?

Response: Thank you for your carefulness. We calculated mean and standard deviation of stress scores of each individual in 8 times follow-ups. Then, 1.5 standard deviation above (or below) the mean of each individual was taken as high (or low) level of stress. In order to make it comparable, we used the mean of high (or low) stress of all the participants to predict the CES-D scores using Hierarchical Linear Model. So, the depression scores in the y-axis are predicted values calculated by high and low stress scores, neither means nor medians. Therefore, error bars could not be presented in Hierarchical Linear Model. The figure we presented in this manuscript is a normal format in analysis of HLM. In previous published papers of HLM, similar figures were all presented without error bars (eg. Yi JY, et al. Insecure attachment as a predictor of depressive and anxious symptomology. Depression And Anxiety. Yang J, et al. The impact of stress on depressive symptoms is moderated by social support in Chinese adolescents with subthreshold depression: A multi-wave longitudinal study. Journal of Affective Disorders.)

To provide a more detail and more accurate description for figure 1, we added the interpretation to figure legend, which have been highlighted with yellow on page 27.

The high/low levels of stressful life events meant 1.5 within-subject standard deviation above/below individual's mean level of stress.

Predicted scores of CES-D were calculated by high/low stress scores using
3. Since the LL females are only 11, how was the classification done with low / high levels of stressful life events? How many females were in these groups? I suggest N for groups should be noted in the figure (or in the figure legend).

Response: Thanks for your suggestion. Since the high levels of life events meant 1.5 within-subject standard deviation above individual's mean level, and low level of stress meant 1.5 within-subject standard deviation below individual's mean level, we did not divided each genotype into two groups but treated it as a whole. According to your suggestion, we added an interpretation to figure legend to show how the high/low levels of stressful life events were classified in the HLM analysis.

Minor revisions:
1. Abstract
"Females with at least one 5-HTTLPR S allele exhibited higher depressive symptoms related to stress”
The authors may want to rephrase this sentence: do they mean that females exhibited more depressive symptoms under stressful situations?

Response: Thank you for your carefulness. We have rephrased it in abstract section as follows, which has been highlighted with yellow on page 3.

Females with at least one 5-HTTLPR S allele exhibited more depressive symptoms under stressful situations.

Introduction
2. The authors refer to the meta-analysis published by Risch et al. [16] in 2009. Since then, other more recent meta-analyses have been published on the subject. I suggest the authors refer to Karg et al., The serotonin transporter promoter variant (5-HTTLPR), stress, and depression meta-analysis revisited: evidence of genetic moderation. Arch Gen Psychiatry.

Response: Thank you for your excellent suggestion. According to your advice, we have replaced Risch et al.’s study with Karg et al’s. Meanwhile, we added the following sentence in the introduction section, which has been highlighted with yellow on page 5.

A meta-analysis published by Karg et al. in 2011 supported that 5-HTTLPR moderates the relationship between stress and depression.

Method
3. "all participants went through thorough neurological and psychiatric screening. Participants who had neurological and current or lifetime Axis I psychiatric disorders according to the DSM-IV" Did the authors use a structured interview to make the diagnoses? A bit more information on how the clinical assessment was done would be useful to the reader.
**Response:** Your suggestions are very useful.

We used the Schedule for Affective Disorder and Schizophrenia for School-Age Children (K-SADS) to make the diagnoses. We added the relevant informations of clinical assessment and K-SADS to procedure and measurements sections as follows, which have been highlighted with yellow on page 8-9.

**Procedure**
Trained researchers who were graduated students from the Second Xiangya Hospital administered both the clinical assessment and questionnaires to the subjects. Neurological physical examination and the interview of the Chinese version of the Schedule for Affective disorder and Schizophrenia for School-Age Children (K-SADS) were conducted one-on-one with each participant outside of class time. Participants who had neurologic diseases, past or current episodes of major depression disorder, manic disorder, bipolar disorder, schizoaffective disorder and schizophrenia were excluded.

**Measurements**
Schedule for Affective disorder and Schizophrenia for School-Age Children (K-SADS)
The K-SADS is a semi-structured clinical interview based on DSM-IV (American Psychiatric Association, 1994) criteria that assesses depressive disorders and schizophrenia in children. The K-SADS has been shown to yield reliable diagnoses of depressive disorders and is frequently used in research on clinical child psychology. In the current study, diagnosticians from the Second Xiangya Hospital were trained to criterion to obtain reliable clinical diagnoses based on DSM-IV criteria. The training program consisted of didactic instruction, conducting practice interviews, and passing a diagnostic exam with an expected minimum score of 85%. The primary investigator of this study held weekly supervision sessions with the diagnosticians and reviewed interviewers' notes and tapes in order to obtain reliable diagnoses. Discrepancies were resolved through consensus meetings and best estimate procedures. Additionally, inter-rater reliability of the K-SADS was tested on diagnosis of 3 students. Nine raters were randomly selected from 21 raters. The Fleiss kappa was 0.73.

**Discussion**
4. The authors say that "future research must test whether 5-HTTLPR is directly associated with depression." Well, there have been a number of studies showing negative associations so perhaps it would be more interesting for future studies to continue to investigate genetic variation in relation life stress, as it is highly unlikely that a single polymorphism has a detectable effect on depression.

**Response:** Thank you for your valuable advice.
We have deleted the sentence "future research must test whether 5-HTTLPR is directly associated with depression" and added a sentence as following, which have
According to the results of the current study, we suggest that there may be no detectable effect of 5-HTTLPR on depressive symptoms of Chinese adolescents.

5. The major limitation of the study is the small sample size. Since the positive association was found only in females, groups were even smaller (n<10 probably). This needs to be emphasized in the discussion and conclusion.

Response: Thanks for your useful advice.

The major limitation of the study is the sample size, which has been emphasized in the discussion on page 18. We added a comment on the limitation in conclusion as following, which have been highlighted with yellow on page 17-18.

*In consideration of the limitation of the small sample size, the conclusions of the current study should be examined in a large sample in future research. If the results can be replicated, they will contribute to exploring genetic and environmental factors in pathological mechanism of depression, and providing definite research evidences for prevention and treatment of depression in Chinese adolescents.*

6. The discussion is well written but rather wordy, I would suggest shortening it a bit, and writing in a more concise, to the point, manner.

Response: Thank you for your excellent suggestion.

According to your helpful advice, we have removed the following sentences in discussion section to make the discussion more concise:

"In other words, the female students carrying at least one 5-HTTLPR S allele exhibited higher depressive symptoms over the 2-year follow-up, during which they experienced more negative life events than their own average levels. In contrast, in male students, the relationship between stress and depressive symptoms was not significantly influenced by 5-HTTLPR. Although the difference was not significant, boys with the LL genotype seemed to exhibit higher depressive symptoms related to stress than those with the SL or SS genotypes."

"Hormonal fluctuation in females, starting in adolescence, may be especially relevant to affective disorder."

"They investigated the cortisol awakening response by exposing subjects to the Trier Social Stress Test,"

"Moreover, in another study, the SS genotype was associated with larger cortisol responses to challenge in females only."

7. Acknowledgements: "Founding" - The authors mean "funding"?

Response: Thank you very much for your carefulness.
We have corrected it in acknowledgments section on page 18.

Additionally, we made some other changes as follows, which have been all highlighted with yellow in revised manuscript.

(1) We added an author "Yu-ping Wang", "Email: yuping_kellen@126.com" on page 1 and added her contribution in authors’ contributions section on page 18.

(2) We revised the conclusions in abstract section on page 4: "In Chinese adolescents, there are gender differences on the interaction between 5-HTTLPR and stress that predict depressive symptoms. The association between stress and depressive symptoms is moderated by 5-HTTLPR in Chinese female adolescents."

(3) We added two subtitles: "Center for Epidemiological Studies Depression Scale (CES-D)" on page 9 and "Adolescent Life Events Questionnaire (ALEQ)" on page 10.

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

Sincerely yours,

Shuqiao Yao, M.D. & Ph.D.
Professor of Psychiatry and Psychology