Author's response to reviews

Title: No interaction between Serotonin Transporter Gene (5-HTTLPR) Polymorphism and Adversity on depression among Japanese Children and Adolescents

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Responses to Reviewer Comments

Reviewer #1: This study examines whether adverse parental environment interacts with 5-HTTLPR polymorphism to predict patient's symptoms of depression in a Japanese pediatric sample. Based on some statistical analyses, the authors concluded that adversity and sex showed a significant main effect despite of no G×E interaction. Today, because G×E interaction is a most crucial controversial issue, a wide range of evidence have been accumulated. Further, the assessment of depressive symptoms in Japanese children and adolescents is of interest to clinicians and researchers in psychiatry, and therefore this study is investigating an important question despite of small sample number. This paper may contribute to the current issue, but seem to have some problems as follows

A. We thank the reviewer for recognizing that “this study is investigating an important question.”

Major problems
1. The aim of the study needs to be clarified before hypothesis is mentioned. The authors should not only mention the aim but also the necessity and novelty of this study to let the readers fully understand the significance of this paper.

A. This is a good point, especially because we had not included that information in the original manuscript. Considering previous research, we hypothesized that 5-HTTLPR is involved in the etiology of childhood depression. No study has tested Japanese children and adolescents. Therefore, the aim of this study is to examine whether opposite G (5HTTLPR genotype) × E (maternal depression) interactions can be confirmed among Japanese children and adolescents. Our additional research findings will contribute to the resolution of inconsistencies in the literature.

We have included the following information in the Introduction section of the revised manuscript.

In the Introduction section:
Considering previous research, we hypothesized that interaction between 5-HTTLPR and early adversity is involved in the etiology of childhood depression. No study has tested Japanese children and adolescents. Therefore, the aim of this study is to examine whether opposite G (5-HTTLPR genotype) × E (maternal depression) interactions can be confirmed among Japanese children and adolescents. Our additional research findings will contribute to the resolution of inconsistencies in the literature.

2. The authors have to mention the reason why there are no significant differences in SES between two groups. Why did you consider the interaction effects between variables such as SES×age, SES×FSIQ, and Sex×age?

A. Thank you for that suggestion. For this study, we recruited age-matched healthy controls who reside in roughly the same area. Fundamentally, the Japanese sample was homogeneous because of the narrow social divide in society. Therefore, we think that no significant differences in SES exist between two groups. However, as shown Table 1, there are significant differences between the two groups in FSIQ, depression scores, and the score of CBCL. Because of the significant main effects of FSIQ and gender, interaction effects are expected to be large.

Added to Results

As shown in Table 1, significant differences were found between the two groups in FSIQ, depression scores, and score of CBCL. Because of the significant main effects of FSIQ and gender, we assume that interaction effects between variables such as SES×age, SES×FSIQ, and gender×age will be large.

3. The authors conducted both binary and multivariable logistic analyses. Please give your views about the reason for those approaches.

A. This is a good point. First, setting “existence of childhood depression” as a categorical dependent variable, we examined what variable made an impact on the onset of depression in subjects. The results of binary logistic analysis revealed the strength of each main effect. Second,
in multivariable logistic analysis, we measured some models' suitability using a data set, leading to a most probable model with a goodness of fit index.

4. The discussion is lengthy. The fourth paragraph and the first half of fifth paragraph are not needed since these don't include consideration relating to this investigation results. The logical plot is quite complicated, so you need to go through a major revision.

A. This is a good point. As you suggested, we deleted the fourth paragraph and the first half of fifth paragraph. Thereby, the logical plot of this manuscript has been clarified.

Minor problems

1. Figure 1 is not needed because the results were written in the text.

A. Thank you for that suggestion. As you have suggested, we deleted it and explained those contents in the text.

2. There are some grammatical errors. English proofreading by professionals is highly recommended.

A. As you have pointed out, our manuscripts have been proofread extensively by professionals. We shall search again for grammatical errors.

Reviewer #2: This is an important area of research and the question is well defined. The methods are appropriate but to account for clinicians being able to generalize these findings some changes are suggested that will clarify issues especially around selection bias. The data is very sound, however, there are some minor errors in describing the data such as the odds ratios. The discussion and conclusion is well balanced. I would
have preferred some more comments about allele frequencies by culture given that this was a somewhat unusually low number of LL's (I know this is mentioned to some extent). The limitation is the homogenous nature of the group. I think the writing is very acceptable.

A. We thank the reviewer for recognizing that “this is an important area of research and the question is well defined.”

Major compulsory

1. Page 9, first line of subjects I note that the patients were referred to a (one assumes) research orientated laboratory and some comments probably should be made about potential referral bias around severity, treatment resistance, possible higher SES etc.

A. Thank you for that suggestion. We have included the following information in the Subjects sections of the revised manuscript.

In the Subjects section:

All the patients were referred from their private pediatric clinics. For that reason, there might be referral bias related to severity despite the drug-naïve condition used for this study.

2. Page 17 results section, second paragraph there was a odds ratio quoted that was in the millions - clearly there is a missing decimal point, similarly further down the paragraph these is another odds ratio of about 1 billion which I suspect is not correct

A. That’s a good point. However, all odds ratios in the original manuscript are correct, as each effect is large. We have corrected the odds ratios from 10 digits to five digits to make them meaningful.

3. Page 9, bottom paragraph I note that the control group gender split is very different to the study group (study group approximately 2 boys to 3 girls whilst control group is 2 boys to 1 girls). This may influence the findings.
A. This is a good point. We have included the following information in the Discussion section of the revised manuscript as one limitation.

In the Discussion section:

Fifth, female predominance was found in the patient group (Fisher exact, \( P = 0.008 \)), which might influence the findings.

In the Introduction section:

Considering previous research, we hypothesized that interaction between 5-HTTLPR and early adversity is involved in the etiology of childhood depression. No study has tested Japanese children and adolescents. Therefore, the aim of this study is to examine whether opposite G (5-HTTLPR genotype) \( \times \) E (maternal depression) interactions can be confirmed among Japanese children and adolescents. Our additional research findings will contribute to the resolution of inconsistencies in the literature.

A. This is a good point. We deleted the sentence “We hypothesized that 5-HTTLPR is involved in the etiology of childhood depression.” In addition, we have included the following information in the Introduction section of the revised manuscript.

In the Introduction section:

I note that severe psychopathology was measured by a proxy measure of a paediatric psychiatrist referral. It would be worthwhile to note if the group had high levels of suicidality and/or comorbidity which would make them more like a typical clinic population.

A. This is a good point. We are sorry that we did not clarify this. The patient group did not have high levels of suicidality or comorbidity. Therefore, we have included that information in the Method section related to subject selection of the revised manuscript.
3. Page 11. It would be useful to know for measures like the WISC-III whether the clinicians administering this was blinded to active or control groups. I note some empirical data is introduced under the heading child behaviour checklist on page 11 and wondered if that should be in the results section.

A. Thank you for that suggestion. Unfortunately, the clinician was not blinded to active or control groups. We have moved the empirical SES method information to the Results section.

4. Page 12, the section ‘early adversity’ I note that no control subjects mother had high early adversity - I thought this environmental descriptor could have been more clearly defined.

A. Thank you for that suggestion. No control subject’s mother had any history of psychiatric problems or exposure to traumatic events / selected from the same community sample / served as no early adversity contrast group. As you suggested, we have included that information in the Method section related to subject selection of the revised manuscript.

5. Page 13, second sentence. I note that the group was ethnically homogenous -this is an important consideration for generalising the findings and could probably be more emphasised in the discussion.

A. Thank you for that suggestion. I have included that information related to subject selection in the Method section and in the Discussion section of the revised manuscript.

6. Page 14 first paragraph I was surprised the observed genotype was similar to the expected Hardy Weinberg equilibrium given that there was only 3% (n=7) LL cases.

A. This is a good suggestion. We are sorry that we did not clarify this. We have corrected this in the Results section by providing much greater detail about the observed genotype. In the Japanese population, LL type of 5-HTTLPR has been reported as rare. Therefore, it might be better to examine the 5-HTTLPR proportion using Fisher’s exact test or Markov chain Monte Carlo
(MCMC) methods. However, some previous reports have described the use of Hardy–Weinberg equilibrium examination empirically (Toyoshima, 2011; Obayashi, 2008). Therefore, we also administered the Hardy–Weinberg equilibrium examination. We have included the following information in the Results sections of the revised manuscript.

In the Results section:

In the Japanese population, LL type of 5-HTTLPR has been reported as rare. Therefore, it might be better to examine the 5-HTTLPR proportion using Fisher’s exact test or MCMC. However, some previous reports have described the use of Hardy–Weinberg equilibrium examination empirically (Toyoshima, 2011; Obayashi, 2008). We also administered Hardy–Weinberg equilibrium examination. The observed genotype distribution was similar to the expected Hardy–Weinberg equilibrium distribution.

Thank you for your attention to this matter. Your comments were very helpful for revision. Your specific comments especially were truly beneficial for us. We hope that the revised manuscript meets with your approval.

Sincerely,

Akemi Tomoda, M.D., Ph.D.

Literature Cited
