Author's response to reviews

Title: The 5-HTTLPR polymorphism of the serotonin transporter gene and short term behavioral response to methylphenidate in children with ADHD

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Author's response to reviews: see over
ITEMIZED ANSWERS TO REVIEWERS:

REVIEWER #1: Maximilian Muenke

Comment 1
I like the way the study design was developed. It is a very straightforward way to assess a pharmacological response for one medication which an average cycle life of 4-6 hours.

Response:
We appreciate this comment.

Comment 2
Page 12: The authors mention that the variable gender is significant when analyzing the genotypes and that for subsequent analyses this will be controlled for. However, on page 11 where they describe the linear mixed-effect model (LMEM) used for their analysis, the gender is not controlled for. What would happen with the CGI scores when controlling by gender? Several studies have shown that gender is strongly associated with ADHD and, among other results, that males are more likely to be diagnosed with the disorder. I would like to see how the results come up once you introduce the gender as a fixed effect in the LMEM.

Response:
We included gender in our analyses (see page 12, results section). Unfortunately, we did not describe this in the “Statistical analysis section” on page 11. Therefore we have added …
The effects of genotype (s’s’, s’l’ and l’l’), gender, treatment (placebo and methylphenidate), treatment order and genotype by treatment interaction were tested using a mixed model analysis of variance.

Comment 3
Along the same lines (comment 2), I consider it important to report the coefficients of the model as well as some diagnostic measures for how well the model fits their data. In the available manuscript, the authors did not present this information. Rather than reporting the F and p-values for every single test (see page 12, for example), I suggest to refer the reader to a table with all the coefficients.

Response:
Few diagnostic tools are available for assessing the adequacy of linear mixed models. Statistics that are sometimes used are Akaike's information criterion (AIC) and the Bayesian information criterion (BIC). Unlike the $R^2$ of traditional linear regression, these statistics cannot be used to ascertain the extent to which the proposed model can explain variation in the outcome. Their use is limited to the
comparison of several models fitted to the same data. Therefore, we did not provide model fit statistics.

Comment 4
One of the main applications of LMEM is to capture the variability within the same sample unit, in this case a child, when it is measured several times. In the present study, each individual represents a sample unit that was measured twice: under a placebo and on medication. In LMEM one of the assumptions is the correlation structure for the sample unit. Did the authors assume any particular correlation structure for the analysis? If yes, which one?

Response:
We used an unstructured covariance matrix for the random effects based on an empirical comparison of several covariance structures (minimizing AIC criteria).

“The effects of genotype (s’s, s’l’ and l’l’), treatment (placebo and methylphenidate), treatment order and genotype by treatment interaction were tested using a mixed model analysis of variance (SAS MIXED procedure, SAS version 6.12, SAS Institute Inc, Cary, NC)"^39^ has been changed to:

The effects of genotype (s’s, s’l’ and l’l’), treatment (placebo and methylphenidate), treatment order and genotype by treatment interaction were tested using a mixed model analysis of variance with an **unstructured covariance matrix** (SAS MIXED procedure, SAS version 6.12, SAS Institute Inc, Cary, NC)^39^

Comment 5
On page 12, “Behavioral response to clinical intervention”, the authors present two p-values, each of them corresponding to different tests. Which cut-off was used for those p-values? (p<0.05, p<0.001, p<0.0001 instead of p<0.00 or is this just a typo?).

Response:
The cut-off used was p<0.001. We have edited the error in the text.

Comment 6
In the Result section the authors mention that no significant difference in IQ was found among groups. Why did not the authors refer to Table 1 for the results? Neither in Table 1 nor anywhere else in the manuscript is there any mention of p-value / statistical tests of IQ measures.

Response:
We have made this change in the text and refer the readers to Table 1.
Comment 7
In the beginning of the “Behavioral response to clinical intervention” the authors write that “Irrespective of genotype, a significant behavioral response during the week of treatment... was observed” and then refer the reader to Figure 1. However, the explanation of their findings both does not correspond to what the p-values are showing and are not clear at all. In addition, it is hard to conclude their statement from Figure 1. Perhaps either a better representation of the data or a different analysis could help to elucidate this point. On the other hand, if the authors found that the genotype is significant, why did they not include this effect to conclude about this on this paragraph?

Response:
We have edited the text and no longer refer the reader to Figure 1. In fact, when the three genotypes are collapsed together, children respond to placebo and methylphenidate in the same way. In order to understand the origin of the interaction, we thought it was important to calculate the change scores to know where the interaction is coming from. When we looked at the s’s’ group, we notice that the interaction is coming from there.

Comment 8
What do the authors mean by “significant genotype by treatment 2-way interaction” on page 12? Do these results correspond to the CGI-Parents or CGI-Teachers score? Also, based on the hierarchical principle of modeling, I would suggest to review the fact of commenting on individual effects of a covariate when a 2-way interaction is present.

Response:
They correspond to the CGI-Parent scores.

Comment 9
On page 13 the authors mention “the Tukey’s Honestly significant differences” and then mention that term again on the next page using an acronym (HSD) not defined anywhere else before.

Response:
We have now defined the acronym on page 13.

Comment 10
What do “the same model of analysis” on page 13 mean? Is that model the same the authors use with the change scores between the week of treatment with placebo and the week of treatment with methylphenidate?

Response:
The same model of analysis as was used for the CGI-P scores.
Comment 11
For the first time in the manuscript the authors mention a correction for multiple tests on page 14. Did the authors use any correction by multiple tests on the other p-values? How would their results change if these corrections were performed?

Response:
Our primary outcome variables were CGI-Parents and CGI-Teachers. However, for side effects, since it was an exploratory analysis, we corrected for multiple testing in this case.

Comment 12
One of the points mentioned by the authors in the discussion was that patients with the s’s’ and s’l’ genotypes tend to exhibit more anxiety disorders compared to patients with the l’l’ genotype. However, from the manuscript I did not see any results supporting this notion.

Response:
We acknowledge this comment and have edited the text accordingly.
REVIEWER #2: Tobias J. Renner

Comment 1:
In general literature cited is quite "antique", more recent work has to be included and discussed! e.g.: gene effect sizes: Faraone and Khan 2006, 5HTT and symptom dimensions: Mick et al. 2009, Oades et al. 2008, Biederman et al. 2008.
Findings of Tharoor et al. 2008, Zeni et al. 2007 on the investigated subject have to be cited and included!

Response:
The literature has been updated and more recent work has been cited.

Comment 2:
Major point: In the introduction there is a substantial error: 5HTT is located on 17q11 not 17p11!!

Response:
This point has now been corrected.

Comment 3:
The introduction is quite lengthy.

Response:
The introduction has been edited.

Methods:
Comment 4:
Was there a wash out period for the cross-over groups after 2 weeks?

Response:
No, there was not.

Comment 5:
Genotyping: for a future evaluation of the functional triallelic variation there should be mentioned, how the distribution of la- and lg-alleles was

Response:
We have provided genotype distribution, from which allelic distribution can be derived.
Results:
Comment 6:
The “effects” of clinical intervention could also be based on the expectancy of the raters (parents and teachers) knowing about that intervention instead of objectively improved behaviours.

Response:
We agree with this comment and in fact, more so for the parents, which can also be seen from the data.

Comment 7:
Major point: suddenly a statistical analysis on the DAT VNTR appears without prewarning in the introduction or methods! To avoid the impression that analyses have been made rather randomly than guided by hypothesis the reason why this is included here should be explained properly and in the proper sections, earlier than the discussion.

Response:
We acknowledge this point and have now mentioned the rationale to look at the DAT1 VNTR in the introduction (Joober R et al. Dopamine transporter 3’-UTR VNTR genotype and ADHD: a pharmaco-behavioural genetic study with methylphenidate. Neuropsychopharmacology. 2007 Jun;32(6):1370-6).

Discussion:
Comments:
Comment 8:
Major point: negative findings by Tharoor et al. 2008, Zeni et al. 2007 have to be discussed!

Response:
Findings from both these studies have been included in the discussion.

Comment 9:
CGI-T does not underline the reported effects. There seems to be only a marginal difference in between the genotype groups and response to MPH.

Response:
We acknowledge this comment and have reported this.

Reference list:
Comment 10:
6): Year is incompletely cited

Response:
The year has now been properly cited.