Author's response to reviews

Title: Functional serotonin transporter gene polymorphisms and anxiety personality traits: new study and meta-analysis on a psychiatrically healthy population.

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Version: 4 Date: 29 October 2010

Author's response to reviews: see over
Brescia, 29th October 2010

Dear Prof. Melissa Norton,

Thank you very much for your kind letter and for having give us the opportunity to present a revised version of our manuscript. We have carefully considered the reviewers’ comments and changed the manuscript accordingly (please see response to reviewers).

All corrections inserted in Abstract, Introduction, Methods and Results sections were written in bold. The discussion section was completely reorganized as suggested and thus it has not been signed in bold.

Once again we would like to thank you and the reviewers for their suggestions that have allowed us to improve the quality of our manuscript. We sincerely hope that the manuscript can be now suitable for publication.

Your sincerely

Prof Massimo Gennarelli
Responses to reviewers:

Reviewer's report 1

In this manuscript, Minelli, et al., report the results of two studies. The first is a candidate gene association study with two functional polymorphisms in the serotonin transporter promoter (5-HTTLPR and rs25531) and anxiety related personality traits as measured by the Harm avoidance (HA) scale of the TCI in 287 Italian adults (59% women). The results of this study suggest that the association between the 5-HTTLPR S allele and increased HA is observed only in individuals with lifetime Axis I disorders, but not in healthy individuals without such diagnosis. The manuscript then reports a meta-analysis of 35 studies that examined associations between 5-HTTLPR and either HA or the personality trait of neuroticism (N). This meta-analysis was intended to either confirm or disconfirm the finding of their earlier analysis that the association of 5-HTTLPR S and elevated HA or N would only be found in samples that did not screen for psychiatric disorders. The results of the meta-analysis confirmed that the 5-HTTLPR association with HA or N was not observed when only studies that screened for psychiatric disorders.

The interpretation of these results was that psychiatric screening is needed in genetic association studies of personality traits to eliminate the potential confound of diagnosis. The study results were also interpreted to support that the S allele of 5-HTTLPR is a risk factor for depression/anxiety spectrum disorders, but not for HA or N in healthy populations.

I found the manuscript to be quite interesting, but I also found that it was not very user friendly, but required significant effort. I am not qualified to provide a detailed critique of the meta-analysis, so I will confine my comments to the association testing. Specific issues are raised below.

Major compulsory revisions:
1. The quality of the written English throughout the manuscript needs to be improved. For example, in the results section of the abstract there are two errors: "we loosed" should read "we lost" and "whatever" should read "whenever". Mistakes like these are found throughout the manuscript.

The manuscript was revised from a English mother language editor and we have corrected the typing errors.

2. Although I am supportive of brief papers, but it seems that this story is too complicated to present in brief. I found it difficult to follow the arc of the narrative at times. In addition, there are opportunities missed for this manuscript to make explicit connections with the empirical literature and to address important issues in the role of 5-HTTLPR in normal and disordered behavior. How do these findings fit with the understanding of the function of S and L alleles? How do these findings contribute to our understanding of the nature of personality traits and mental illness? Are depression and anxiety disorders qualitatively different from normal variation in anxiety and neuroticism?

We answered to these comments:
- recently it has been proposed the hypothesis about a role of the serotonin transporter gene not directly in the MDD susceptibility but rather in the some features of the pathology such as the response/resistance to antidepressant treatment (Bonvicini C et al 2010; Zandi PP and Judy JT 2010; Horstmann S and Binder EB 2009; Serretti A et al 2007), or the interaction with the stressful life events, given the robust correlation between these events and risk of developing depressive symptoms (Wankerl M 2010; Caspi A 2010, Goldman et al 2010; Uher et al 2010).
These findings have been inserted in the discussion section and the references are reported in the “references” section.
- Furthermore, some personality traits such as HA occur across several diagnostic categories beyond the depression, i.e. obsessive compulsive disorder (Alonso P. et al 2008; Lyoo I.K. et al 2001), eating disorder (Fassino S. et al 2004), borderline personality disorder (Barnow S. et al 2007), and further work should be made to clarify the relationship between personality traits and single disorders. Thus, to date it is not possible argue differences in anxiety personality traits between depression and anxiety disorders.

3. Issues regarding the statistical power to detect effects need to be explicitly addressed. The authors need to demonstrate conclusively that the loss of the association when the disordered subjects are removed is not a function of reduced statistical power
We have inserted the statistical power sample analysis in the page 9 line 4 Methods section with the result that the association study showed a power > 80% on all samples analyzed.

4. The potential impact of gender needs to be addressed. Gender was included as an independent variable in the analyses, but no gender results are reported.
We agree with the reviewer and thus we have added the lack of significant Gender effects in all analyses (page 10 line 18).

5. In general, the Discussion section needs to be more thorough in its efforts to interpret these results and put them in to context.
We have reorganized completely the discussion in order to clarify our results and our findings.

Minor Essential Revisions:
1. The title should be more informative as to the specific markers assessed and to the results.
We have changed the title in order to emphasize our results in “The influence of psychiatric screening in healthy populations selection: a new study and meta-analysis of functional 5-HTTLPR and rs25531 polymorphisms and anxiety-related personality traits”
2. Essentially, the reported effect can be discussed as a case of moderation. In other words, Axis I diagnosis moderates the association between 5-HTTLPR and HA. Such an interpretation needs to be more thoroughly explored in the discussion. What does this study contribute to knowledge about the relations among 5-HTTLPR, HA and diagnosis?
This study confirms the lacking of an association of the functional serotonin transporter gene polymorphisms and anxiety traits in healthy subjects screened through a structured psychiatric interview. Contrarily, although a small sample size, the association is observed in the “disordered” group with lifetime DSM-IV Axis I disorders.

3. How do these findings fit in with the literature on GxE interactions (5-HTTLPR x life stress) in the etiology of depression?
As already reported above, we have discussed this finding in the discussion section.

4. It is not clear to me that all of the statistical analyses are necessary for the 5-HTTLPR HA association test. Why not just a single ANOVA and post-hoc test of interaction effects? Why do the subsequent ANCOVA reported in the 2nd paragraph of the results?
As often reported from literature (see meta-analysis Miettunen J. et al 2008), HA correlates with age and gender. Also in our sample, HA showed significant association with the variable Age ($r = 0.16; p < 0.01$) and Gender ($r = -0.22; p < 0.01$). For this reason thus we have carried out the ANCOVA reported in the 2nd paragraph.
5. Error bars (s.e.) should be included on figure 1.

**We have deleted figure 1 as suggested from reviewer 3.**

6. What do these results say about the influence of rs25531?

**We have added in the discussion section page 17 line 10 the influence of this rs on the modulation of 5-HTTLPR where L₃ haplotype has lower transcriptional efficacy as well as the S allele. Therefore, in STUDY I we conducted association analyses for 5-HTTLPR/rs25531 to investigate the influence of rs25531. The results showed the association with HA in the “disordered group”. In light of these data, we can speculate that also the genotyping of the both functional polymorphisms (5-HTTLPR and rs25531) and the haplotypes analysis could be taken into account in relation to anxiety-related personality traits.**

7. It would be useful to see the ANOVA tables and the effect sizes (i.e. proportion of variance explained) should be provided.

**We did not add effect sizes in the tables for not confusing the readers with too much data on tables.**

The Partial Eta Squared Indices, indicated in the brackets, for significant results are:

- Carriers L vs SS in the whole sample $p = 0.02 (0.02)$; Carriers L vs SS in the “disordered” group $p = 0.05 (0.07)$; (L’L’ + L’S’) vs (S’S’) in the whole sample $p = 0.05 (0.02)$; (L’L’ + L’S’) vs (S’S’) in the “disordered” group $p = 0.03 (0.09)$.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

The manuscript was revised from a English mother language editor.

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.
Reviewer's report 2

This study investigates the association between 5-HTT-LPR short allele and anxiety traits (assessed by harm avoidance) in 229 healthy subjects and 38 with a lifetime history of disorder. A meta-analysis of this association in previous studies is included. The conclusion is made that there is an association in healthy but not in disorder. This conclusion is not well supported by the data, given the very much smaller number of subjects with a disorder. It is just as feasible that the lack of association reflects the lack of power in the small sample of 38.

There is also a lack of information about current diagnostic status of this sample. Replication of short allele associations is needed. It would be valuable for the authors to include at least as large a sample of clinical patients to test their claim.

Our sample was constituted of 287 voluntaries of which 229 “healthy” subjects and 55 named “disordered” group. The power analysis showed a value > 80%.

The results obtained were as following: when we performed the analyses on the whole sample of 287 volunteers, an effect on the susceptibility to HA were found for both the SS genotype and S’S’ haplotypes. However, because the screening assessed by M.I.N.I. showed the presence in this sample of 58 volunteers affected by Axis I disorders, we regrouped subjects in two different groups. One constituted of subjects without Axis I disorders named “healthy” group and another of the 55 subjects affected from depression and/or anxiety disorders (named “disordered group”). Three subjects affected respectively by Bipolar Disorder, experienced alcohol and substance abuse were excluded. Thus, we have verified the possible influence of the variable “groups” (“healthy” and “disordered”) on the genotypes. The results have evidenced a significant interaction between genotypes and groups (5-HTTLPR, p = 0.03 and 5-HTTLPR/rs25531 L’L’ + L’S’ vs. S’S’; p = 0.02) and consequently, we have conducted the analyses separated for both groups. The data obtained did indeed confirm that in “healthy” group the significant effect of SS genotype and S’S’ haplotypes was lost but it remained in the “disordered” group. The results on 5-HTTLPR meta-analyses and anxiety-related traits in the whole sample confirmed the association of SS genotype with higher anxiety-related traits scores in Caucasoid, but whatever we executed the analysis including only the studies using a structured psychiatric screening, no association was found.

However to be more thorough in the interpretation of these results and put them in to context, we have completely reorganized the discussion and improve the introduction section.

Concerning the last comment, the main goal of our work was to underline the importance of a psychiatric screening on the controls selection. We agree with the referee about the too small sample size of the “disordered” group however the power analysis showed a value > 80% and thus we can take in account the results obtained.

Level of interest: An article of limited interest
Quality of written English: Not suitable for publication unless extensively edited The manuscript was revised from a English mother language editor.
Statistical review: Yes, and I have assessed the statistics in my report.
Reviewer’s report 3

Title: Functional serotonin transporter gene polymorphisms and anxiety personality traits.
New study and meta-analysis on a psychiatrically healthy population.

The present paper raises two different studies about the relationship between two polymorphisms of the serotonin transporter gene and anxiety personality traits. In general, the paper has good quality and adds useful information to the literature, however, I think that some aspects of the organization and discussion may be improved.

First of all, the title is not right. Note that some analysis with the new sample and meta-analysis are conducted on diagnosed samples.

We have changed the title in order to emphasize our results in “The influence of psychiatric screening in healthy populations selection: a new study and meta-analysis of functional 5-HTTLPR and rs25531 polymorphisms and anxiety-related personality traits”

Secondly, organization of the paper is confused. I think it will be easier to understand the paper if new study and meta-analysis were named as Study 1 and study 2, respectively, and, therefore, Method and results sections for each study were separated. With the present format, it is difficult to follow both studies. Besides, the study 1 may be used to support the meta-analysis.

We have reorganized the paper stratified for study 1 with the respective Methods and Results sections and study 2 with respective Methods and Results as you suggested.

On the other hand, I have some comments about other aspects of the paper. The introduction is brief but adequate. I have one comment only. Authors speak in the entire section about healthy and psychiatric samples, however, they change the names for controls and screened samples in the last paragraph. They should retain the nomenclature of the entire section. In the method section of the first study, authors state that “only healthy volunteers.............were enrolled in the study” (p. 6). But this statement is false since subjects with diagnosis of axis I were also analysed.

We have corrected as you suggested. In particular we have named the subjects without Axis I disorders as “healthy” and those with these disorders as “disordered” in the whole manuscript.

The next sentence is “The personality traits were assessed by the Italian version of M.I.N.I.” (p.6). I guess this is a mistake since, as far as I understand, personality traits were assessed by the Italian version of the TCI. Besides, the reference 27 should be associated to the publication of the Italian version of the TCI, not the American one.

We have corrected the mistake and have replaced the reference with Martinotti G. et al., 2008 (page 8 line 13).

Analyses were well conducted and presented, but I do not understand why group variable is introduced as a covariate since analyses were further conducted separately for both samples.

We have carried out an ANCOVA using HA score as the dependent variable, groups (“healthy” N=229, and “disordered” N=55), genotypes, and sex as independent variables, and age as a covariate for both the 5-HTTLPR and the estimated/phased haplotypes. Because we have obtained a significant interaction between genotypes and groups (5-HTTLPR, p = 0.03 and 5-HTTLPR/rs25531 L’L’ + L’S’ vs. S’S’; p = 0.02), we have conducted the analyses for both groups separately.

Also, figure 1 could be deleted since all relevant information is reported on table 1.

We have deleted the figure 1 as you suggested.

In regard to the discussion section, the mixture of both studies make hard to understand this section. With respect to the first study, authors suggest the relevance of employing more stringent inclusion/exclusion criteria. But, it is not clear if they advise for not including
psychiatric patients or they call for including them in this kind of genetic studies. In fact, this is the most serious concern I have about the paper. Authors do not explain the reason for reported differences between healthy and psychiatric samples. Note that the pattern of HA means for healthy subjects is the opposite of that found for the psychiatric subsample. This is strange since the expected pattern will be the same for both samples. Authors should discuss the reason for such result. Let me to advance a tentative explanation. In the meta-analysis by Munafo et al., (2003), they suggest that extreme groups analysis may give more chances of finding significant associations. They are theoretical and statistical reasons to expect that. I think that including psychiatric samples implies to work with the entire distribution of the trait. On the contrary, the sample distribution may be truncated and, therefore, reducing the statistical effects. This bias may be stronger on volunteer and elder samples as the one analysed in the new study. So, psychiatric sample would be the right side of the phenotype distribution. If you look at the means of the groups, you should note that the averages are quite larger in the psychiatric sample than in the healthy one. Considering the meta-analysis, I was not be able of understand if authors conclude that there is an association between 5-HTTLPR and NEO or TCI. Please, clarify the redaction of the discussion about the meta-analysis.

Finally, it is somewhat contradictory that 5-HTTLPR was not involvement in anxiety related traits variability but it was confirmed (supported?) to be a risk factor for depression/anxiety spectrum disorders. This contradiction is difficult to sustain since it is well known that anxiety traits are strongly linked to the depression/anxiety disorders. If authors retain both conclusions, they should raise some explanation for such contradiction.

**In order to answer to the different comments, we have reorganized the whole discussion.**

References:


**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.
Editorial Requests:

Authors' contributions - Please include an Authors’ contributions section before the Acknowledgements and Reference list.
We have inserted the “Authors’ contributions” section as indicated.

Title page - The full names, institutional addresses, and e-mail addresses for ALL authors must be included on the title page. The corresponding author should also be indicated.
We have inserted the full names, institutional addresses, and e-mail addresses for all authors in the title page.

Meta-analysis - Please ensure that your manuscript adheres to the MOOSE (meta-analyses of observational studies) guidelines which can be found at: http://www.consort-statement.org/resources/downloads/other-instruments/
Our manuscript adheres to the MOOSE guidelines (Stroup DF et al., 2000)