Author's response to reviews

Title: Association of TPH-1 and TPH-2 genes with suicidal behavior: A systematic review and meta-analysis

Authors:

Thelma B González-Castro (thelma.glez.castro@gmail.com)
Isela E Juárez-Rojop (iselajua22@yahoo.com.mx)
Lilia López-Narváez (dralilialonar@yahoo.com.mx)
Carlos Tovilla-Zárate (alfonso_tovillaz@yahoo.com.mx)

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Author's response to reviews: see over
Dr. Laura Mandelli
Editor of BMC Psychiatry

Thank you very much for giving us the opportunity of revising our manuscript. Also, we greatly appreciate the time and effort the reviewers spent in our manuscript. We would like to re-submit the paper entitled: “Association of TPH-1 and TPH-2 genes with suicidal behavior: A systematic review and meta-analysis” (MS: 1546893095111544) by Thelma González et al. as a Research Article in BMC Psychiatry. My colleagues and I believe this revision has been markedly improved. The answers to your remarks together with point to point clarifications are listed below; these include the changes asked and kindly suggested by the referees. The authors are indebted to the referees for improving the quality of the present article with their remarks.

Referee 1: Zainab Samaan

External Reviewer’s Comments

Association of TPH-1 and TPH-2 genes with suicidal behavior: A systematic review and meta-analysis” González-Castro Thelma Beatriz, Juárez-Rojop Isela, López Narváez María Lilia, Tovilla-Zárate Carlos Alfonso. The authors conducted a systematic review and meta-analysis of studies investigating the association between TPH genes and suicidal behavior and concluded that a genetic variant of TPH-1 is associated with suicidal behavior.

General comments and essential revisions
1. The manuscript requires English language revision. Several sentences and paragraphs should be re-phrased. For example: **Abstract**

a) “Nowadays is widely acknowledged that suicidal behavior” (Page 2; Paragraph: 1; Line: 2). This was corrected.

b) “so we performed a meta-analysis to get a better insight into this matter.” (Page 2; Paragraph: 1; Lines: 6-7). This was also corrected.

c) “also we performed a sub-analysis by Caucasian and Asian populations” this should be a subgroup analysis. We agree. This was corrected (Page 2; Paragraph: 2; Lines: 11-12).

d) “The study provided evidence stating that A218C/A779C TPH-1 variants may be a risk factor to present SB” this is not clear as to what “present SB” refers to? We agree. This was corrected (Page 2; Paragraph: 3; Lines: 16-17).

e) “With regard to G-703T/A-473T/G19918A TPH-2 variants, our up-to-date meta-analysis did not detect any significant.” This sentence seems incomplete. “any significant” association between genetic variants and SB? We agree. This was corrected (Page 2; Paragraph: 3; Lines:18-20).

2. Examples from **background** section: “As a result, the G-703T polymorphism has been reported to be higher in suicidal depressed patients,” higher frequency in patients compared to healthy controls? Several other examples of linguistic nature exit throughout the manuscript. We agree. The text was checked (Page 4; Paragraph: 1; Lines: 11-13).

2. In addition, many statements in the manuscript are not referenced, for example, background, 1st paragraph “Worldwide, suicidal behavior (SB) has become a public health issue that accounts for almost one million deaths annually and is the leading cause of death among individuals between 15 and 44 years old.”, “Moreover, the evidence from these several genetic studies indicates that dysfunctions of the central serotonergic system are involved in the pathogenesis of suicidal
behavior.” Authors must provide appropriate references for every factual statement presented throughout the manuscript. We agree. This issue has been corrected throughout the manuscript.

**Major Compulsory Revisions**

1. **Background:** The research question at the end of the background should be revised. The authors should outline their research question explicitly in a PICOT (population, intervention, comparison group, outcome, time-frame) format. Outlining the question in this format will provide the reader with an explicit sense of what this review will be investigating. In addition, an objectives section at the end of the introduction may help readers to better follow the format of this review. We agree. We added the research question and clarified our aims. (Page: 4; Paragraph: 1; Line: 23 and Page: 5; Paragraph: 2; Lines: 1-11).

2. **Methods:** Authors should indicate whether or not a protocol was developed for this study and if so, where it can be accessed. This is an important part of a systematic review and ensures that the methods are transparent and the objectives are decided a priori. We agree. We added this point at the beginning of the Methods section (Page: 5; Paragraph: 3; Line: 13).

Authors should specify if any constraints were placed on their searches, including temporal or language constraints, and include reasons for why or why not they were used. It would also be helpful to include reasons for excluded studies (figure 1, excluded 8 studies). Also, if there were missing data in the studies, please specify how it was handled. Lastly, the outcome of suicidal behavior is very broad, it would be helpful to describe exactly what this outcome entails and how it is defined across studies. Overall the search strategy is not clearly described with detailed search terms, there is also no mention of duplicate articles (for example searching Medline and PubMed will generate many duplicates), the flow diagram is not detailed, box one shows articles retrieved from EBSCO? And PubMed. The title of figure 1 should also be changed as per PRISMA
guidelines; it is not “stages of meta-analysis”. The main serious limitations in the methods section are the search strategy. The authors have made an OVID MEDLINE search as well as a PubMed search; however, MEDLINE search encompasses a search of PubMed. In addition, the outline of the search strategy is very limited and could not be replicated. Different databases have different MESH terms. Please see an example search strategy per database in Figure 1 below. The search should be transparent and replicable. Please also include, if possible, a measure of inter-rater agreement (i.e. Kappa statistic) for the screening of titles, abstracts, and full-text extraction. We agree. We modified several points in the manuscript to show more specific information and we changed Figure 1 according to PRISMA criteria (Page: 6; Paragraph: 3; Lines: 21-23). The Kappa statistic was not performed.

3. Results: Figure 2 does not add to the findings; I suggest removing it. The authors used the Newcastle Ottawa Scale to assess quality of studies however results from this were not reported. It would be helpful to include a results table to present the scores of studies. In addition, the authors did not explore risk of bias adequately. When addressing risk of bias, it would be useful to use the GRADE approach and assess the risk of bias and discuss your confidence in the estimates. Suggest creating a GRADE table (Guyatt G, Oxman AD, Kunz R, Brozek J, Alonso-Coello P, Rind D, Devereaux PJ, Montori VM, Freyschuss B, Vist G, Jaeschke R, Williams JW Jr, Murad MH, Sinclair D, Falck-Ytter Y, Meerpohl J, Whittington C, Thorlund K, Andrews J, Schunemann HJ. GRADE guidelines 6. Rating the quality of evidence – imprecision. (JCE 2011). The GRADE working group has created a method of determining the confidence in the estimates by using the risk of bias assessment in addition to the assessment of meta-analysis precision to rate the quality of the evidence (moderate, high, low), based on study design and other criteria. We agree. We added two tables as supplementary material to address this point. (Supplementary material)
The Forest plot figures: please provide sample size for each study and revise the figures output as the point estimate should reflect the sample size for each study (Discretionary revisions). We agree. We modified all forest plots.

The conclusion of association between TPH-1 and SB should be interpreted with caution as it can only be seen in the fixed effect model. A discussion on the possible reasons for these findings including statistical errors should be presented. The authors performed multiple testing using multiple models and several genetic variants, an adjustment for multiple testing should also be considered. This may change the overall conclusion of this study. We agree. We included some information in the discussion section (Page: 13; Paragraph: 1; Lines: 8-11).

4. Discussion: The subgroup analyses explored in this paper were not reported in the methods section a priori, this makes these estimates look largely results driven (or exploratory). It would be important to mention in the background the reasons for subgroup analyses. We included some information to clarify our methods. (Page:7; Paragraph: 2; Lines: 20-21 and Page: 5; Paragraph: 1 ; Lines: 5-7).

Referee 2: Pao-Yen Lin

Major compulsory revisions:

1. Authors did not clearly describe why this meta-analysis needs to be done in the introduction, since there are many well done analyses that have been published in the past few years. We agree. We justified our aims in the background section (Page: 4; Paragraph: 2; Lines: 21-23 and Page: 5; Paragraph: 1; Lines: 1-5).

2. Definition and sub-classification of suicide behaviors are not considered in this analysis. We added information in the discussion section as part of the limitations of the study. (Page: 15; Paragraph: 1; Lines: 1-2).
3. Did authors consider factors that may modulate the association, such as gender, age, publication year, and so on? Indeed. We added information in the discussion section in the part where the limitations of the study are mentioned (Page: 14; Paragraph: 3; Lines: 24 and Page: 15; Paragraph: 1; Lines: 1-3).

4. Did authors consider the influence of co-morbid mental illnesses of the SB subjects? We clarified this information in the discussion section when we mentioned the limitations of the study (Page: 15; Paragraph: 1; Lines: 5-6).

5. It is not clear from Figure 3-8 authors selectively reported forest plots and funnel plots for TPH2 gene variants, but none for TPH1. We agree. We included plots for TPH-1 in Additional file 2.

6. In discussion, it would be better to provide more neurobiological explanation of how TPH protein regulates susceptibility of SB through possible endophenotypes, such as impulsiveness, learned helplessness. We clarified the information in the discussion section as part of the limitations of the study (Page: 15; Paragraph: 1; Lines: 5-6).

Minor essential revisions:
1. Line 6-7 of first paragraph of introduction: “…dysfunctions of the central serotonergic system are involved in the pathogenesis of suicidal behavior…” should be supported by citations. This was checked.

2. There is no one citation about the characteristics and functions of association of TPH1 with clinical phenotypes or neurobiological importance. There should be a large amount of literature. We included some extra information (Page: 3; Paragraph: 1; Lines: 13-17).

3. Grammatical errors should be corrected. They were corrected.

Referee 3: Clement Zai

Major compulsory revisions:
Overall it is a potentially interesting meta-analysis. However besides the grammatical/typographical errors and awkward sentences throughout the text and the Tables, there are a large number of important issues that need to be addressed before the manuscript can be properly reviewed:

1. The authors need to include more recent suicide genetics reviews in the Introduction. We included more references in the background section.

2. The authors should provide references for the PRISMA criteria and the Newcastle-Ottawa Assessment Scale. We agree. This was included in the methods section (Page 5; Paragraph: 3; Lines: 13-15 and Page 8; Paragraph: 1; Line: 25).

3. Please double-check the SNP IDs (e.g., rs11178997). This was checked (Page 4; Paragraph: 1; Line: 10).

4. There need to be more stringent checking of the data included in the meta-analysis. There are a large number of issues with the data. For example (the a-h list below may not include all issues):

a. For the Zhou et al (2005) paper, the authors should include the African, American and Southwestern American Indian samples in the all-population meta-analysis. We agree. These populations were included in the analysis results.

b. The use of data from the Haghighi et al (2008) paper may not be appropriate because the case group has either suicide or depression, so some “cases” might not have been suicide cases. We agree. This paper was excluded from the study.

c. The controls for Zill et al (2004) and (2007) overlap. This was checked.

d. The samples from Rujescu et al (2002) and (2003) appear to overlap and need to be double-checked. Also, genotype data for A779C was not available from the Rujescu et al (2003) paper; what is the source of this data? These points were corrected.
e. The case sample and genotype/allele counts for Bellivier et al (1998) and Abbar et al (2001) studies appear to be double of what they should be. This was checked.

f. The data for the Pooley et al (2003) study appears to be for A779C and not A218C. Please check. We agree. This was corrected.

g. The data among the Croatian studies (Jernej et al, 2004; Stefulj et al 2005; Stefulj et al, 2006) may be overlapping. The frequencies of the minor/major alleles look flipped. This point was checked.

h. The A218C and A779C genotypes for Saetre et al (2010) suicide cases were flipped, and the genotype counts for the controls need to be checked. This point was checked.

6. I am not familiar with the use of virtual controls, thus I would recommend a statistical review. I am concerned with the potential bias that may arise from this approach. Thank you.

7. The authors need to specify the suicidal behaviour: whether it is general suicidal behaviour, suicide attempt history, suicide completion, or suicidal ideation, as well as underlying psychiatric diagnoses if available. We added some information in Tables 1 and 2.

8. The author must provide sex ratio for each study and, if possible, do a separate meta-analysis for males and females. We agree, we wrote some information in the discussion section in the lines dealing with the limitations (Page: 14; Paragraph: 2; Lines: 24 and Page: 15; Paragraph: 1; Lines: 1-3).