

PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Association between HTR2C gene variants and suicidal behaviour: A protocol for the systematic review and meta-analysis of genetic studies
AUTHORS	Tovilla-Zárate, Carlos; González-Castro, Thelma; Juárez-Rojop, Isela; Genis-Mendoza, Alma; López-Narváez, María

VERSION 1 - REVIEW

REVIEWER	Raffaella Calati Department of Biomedical and NeuroMotor Sciences University of Bologna Bologna, Italy
REVIEW RETURNED	12-May-2014

GENERAL COMMENTS	<p>The present study aimed to systematically review and meta-analyze studies investigating the association between serotonin 2C receptor gene (5-HTR2C) and suicidal behaviour. In particular, it aimed to focus on 5-HTR2C rs547536, rs2192372, rs4272555, rs6318 and rs2428707 polymorphisms. Although the goal is a valuable one, the protocol shows some consistent critical issues, represented by: 1) the lack of a clear definition of the rationale of the study (e.g., the reason for the selection of 5-HTR2C among all serotonergic genes; the mention of Mexican population is not clear) and of a more detailed methodological description (what about sensitivity analyses and meta-regression?); 2) the probable low number of the studies that could be included in the meta-analysis and the consequent small sample size; 3) the high heterogeneity of the sample (e.g., gender, age, ethnicity and psychiatric diagnosis should be considered in the data analysis); 4) the fact that psychiatric patients with suicidal behaviour were compared with healthy subjects only, without considering psychiatric patients with no suicidal behaviour.</p> <p>Authors should modify the manuscript according to the following suggestions.</p> <p>The manuscript should be carefully revised by a native English speaker.</p> <p>In the abstract section and also in the whole paper, Authors should firstly focus on the systematic review and then on the meta-analysis.</p> <p>ABSTRACT Authors should underline that they want to perform a systematic review and a meta-analysis of genetic studies investigating the association between 5-HTR2C and suicidal behavior. They should firstly focus on the systematic review and then on the meta-analysis, also resuming inclusion/exclusion criteria.</p>
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INTRODUCTION

Authors wrote: "Suicidal behavior has been investigated around the world, most of the studies have been based on Caucasian and Asian population, but in Latin American countries, there is no information related to this problem". This sentence is not clear. As Authors want to perform a review and meta-analysis on previous studies investigating the association between 5-HTR2C and suicidal behaviour in Mexican population, some literature data are obviously required. Please, specify. However, this could obviously lower the sample size.

Moreover, Authors should pay more attention to logical connection between sentences. In particular, the second paragraph is not well connected to the first one. Authors should firstly provide some general information about the association between the serotonergic system and suicidal behaviour and then focus on 5-HTR2C. They also wrote: "Since anxiety and mood disorders, as well as impulsiveness, are important suicide risk factors, the changes in serotonin receptor 2C could be linked to suicide". Since other serotonin receptors were found to be associated with anxiety or mood disorders (e.g., 5 HTR1A), why do Authors want to focus on 5-HTR2C gene? This choice should be better explained. In addition, why did Authors mention impulsiveness as suicide risk factor? Has impulsiveness been associated with 5-HTR2C as well? Please, specify.

AIMS AND OBJECTIVES

Why did Authors report that they aimed to investigate the association between 5-HTR2C and suicidal behaviour in Mexican population? Moreover, they should also specify why they want to focus on rs547536, rs2192372, rs4272555, rs6318 and rs2428707 polymorphisms. Are they the only available ones? And how many studies are available for each. This could be a strong limitation for the meta-analyses.

METHODS/ANALYSES

Identification and selection of publications

Inclusion and exclusion criteria should be described more in detail, separately considering the review and the meta-analysis. A clear definition of what Authors mean with suicidal behaviour should be provided (e.g. suicidal ideation, planning, attempt, completed suicide?).

Please, explain the reason for the sentence "The dates of publication of the searched papers will be from 2003 to 2014": why 2003? Please, indicate the month as well.

As for the meta-analysis, a study selection procedure should be provided.

Data extraction

The specific investigated SNPs should be reported, as well as the inventories used to define the suicidal behaviour.

Data analysis

Due to the location of the 5-HTR2C gene on the X chromosome, the analyses should be performed separately considering males and females. Moreover, Authors should compare, as sensitivity analysis, suicide attempters with the same psychiatric diagnosis to healthy controls (not only suicide attempters in general) in order to reduce the influences of psychopathology on the observed differences. A meta-regression including age and ethnicity should be further performed. Finally, what about the possible presence of linkage disequilibrium among the investigated SNPs and haplotypic

	<p>analyses?</p> <p>DISCUSSION Authors should consider the suicidal behaviour according to the stress-diathesis model, and discuss the results also on the basis of it. Please, report strengths and limitations as well.</p> <p>Minor remarks: Abstract I suggest to consider the 5-HTR2C as involved in suicidal behaviour and not as cause Discussion Authors wrote: "Those who attempt suicide and survive, apart of having serious injuries (such as broken bones, brain damage, or organ failure), very often develop depression and other mental health problems, as consequences". This sentence is not correct. Suicide attempters do not develop depression or other mental health problems; the latter may be worsen by suicide attempts. Authors wrote: "serotonin neurotransmission system has received the most consideration as a candidate gene in suicidal behavior studies due to its role in mood regulation and because its function is altered in suicide". Maybe Authors wanted to write "as a candidate system" and not gene.</p>
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VERSION 1 – AUTHOR RESPONSE

Reviewer: Raffaella Calati

The present study aimed to systematically review and meta-analyze studies investigating the association between serotonin 2C receptor gene (5-HTR2C) and suicidal behavior. In particular, it aimed to focus on 5-HTR2C rs547536, rs2192372, rs4272555, rs6318 and rs2428707 polymorphisms. Although the goal is a valuable one, the protocol shows some consistent critical issues, represented by:

1) The lack of a clear definition of the rationale of the study (e.g., the reason for the selection of 5-HTR2C among all serotonergic genes. Response. We thank Raffaella Calati for this comment. We have clarified in the introduction (Page 5, lines 4-9).

- the mention of Mexican population is not clear). Response: We will use any population world wide; to be clear about it, we have modified our aims (Page 5, lines 12-16).

-and of a more detailed methodological description (what about sensitivity analyses and meta-regression?) Response: We agree and have added the sensitivity analyses in the Methods section (Page 8, lines 7-9).

We do not plan to perform a meta-regression as we do not have the right software available.

2) The probable low number of the studies that could be included in the meta-analysis and the consequent small sample size. Response: We have clarified this in limitations and discussion (Page 9, lines 19-23).

3) The high heterogeneity of the sample (e.g., gender, age, ethnicity and psychiatric diagnosis should be considered in the data analysis). Response. We have clarified this in limitations and discussion (Page 10, lines 1-2).

64) The fact that psychiatric patients with suicidal behavior were compared with healthy subjects only, without considering psychiatric patients with no suicidal behavior. Response: We consider this an excellent comment and will include reports that used psychiatric patients with no suicidal behavior (Page , lines 10-12).

Authors should modify the manuscript according to the following suggestions:

a- The manuscript should be carefully revised by a native English speaker. In the abstract section and also in the whole paper. Response: Thank you, we have done so.

b- Authors should firstly focus on the systematic review and then on the meta-analysis. Response: Yes, we are planning to do it and have made it clear in the aims section (Page 5, lines 12-16).

ABSTRACT Authors should underline that they want to perform a systematic review and a meta-analysis of genetic studies investigating the association between 5-HTR2C and suicidal behavior. They should firstly focus on the systematic review and then on the meta-analysis, also resuming inclusion/exclusion criteria. Response: Yes, we will focus on the systematic review and then the meta-analysis; we have modified the abstract to make it clear (Page 2, lines 9-12).

c) INTRODUCTION Authors wrote: "Suicidal behavior has been investigated around the world, most of the studies have been based on Caucasian and Asian population, but in Latin American countries, there is no information related to this problem". This sentence is not clear. As Authors want to perform a review and meta-analysis on previous studies investigating the association between 5-HTR2C and suicidal behavior in Mexican population, some literature data are obviously required. Please, specify. However, this could obviously lower the sample size. Response: We want apologize for this sentence; we have rewritten this part of the introduction and removed the useless information. We will review world wide reports and have made it clear in the aims and objectives (Page 5 , lines 12-16).

d) Moreover, Authors should pay more attention to logical connection between sentences. In particular, the second paragraph is not well connected to the first one. Response: We have modified the whole manuscript and correctly connected every paragraph.

f) Authors should firstly provide some general information about the association between the serotonergic system and suicidal behavior and then focus on 5-HTR2C. Response: We have added this information in the introduction (Page 4, lines 13-16).

g) They also wrote: "Since anxiety and mood disorders, as well as impulsiveness, are important suicide risk factors, the changes in serotonin receptor 2C could be linked to suicide". Since other serotonin receptors were found to be associated with anxiety or mood disorders (e.g., 5 HTR1A), why do Authors want to focus on 5-HTR2C gene? This choice should be better explained. Response: We have rewritten this section in order to clarify it in the introduction (Page 4, lines 16-21).

h) In addition, why did Authors mention impulsiveness as suicide risk factor? Has impulsiveness been associated with 5-HTR2C as well? Response: Yes, the 5-HTR2C has been associated with impulsiveness; we have clarified this in the introduction (Page 4 , lines 19-21).

i) AIMS AND OBJECTIVES Why did Authors report that they aimed to investigate the association between 5-HTR2C and suicidal behavior in Mexican population? Moreover, they should also specify why they want to focus on rs547536, rs2192372, rs4272555, rs6318 and rs2428707 polymorphisms. Are they the only available ones? And how many studies are available for each. This could be a strong limitation for the meta-analyses.

Response: We apologize for the misunderstanding; we will study any population around the world and have made it clear in the aims and objectives. (Page 5, lines 12-16)

k) METHODS/ANALYSES Identification and selection of publications. Inclusion and exclusion criteria should be described more in detail, separately considering the review and the meta-analysis.

Response: We deeply appreciate the reviewer's suggestion and have detailed each analysis. (Page6 , lines 6-12).

m) A clear definition of what Authors mean with suicidal behavior should be provided (e.g. suicidal ideation, planning, attempt, completed suicide?). Response: We have added the definition and forms of suicidal behavior in the introduction (Page 4, lines 4-10).

n) Please, explain the reason for the sentence "The dates of publication of the searched papers will be from 2003 to 2014": why 2003? Please, indicate the month as well. Response: To our knowledge, the first study of an association between HTR2C was reported in 2003; nevertheless, we have deleted this sentence.

o) As for the meta-analysis, a study selection procedure should be provided. Response: We agree

and have added figure 2 to exemplify the study procedure.

p) Data extraction. The specific investigated SNPs should be reported, as well as the inventories used to define the suicidal behavior. Response: We have done this (Page 6 , lines 21 and page 7 line 1 and page 6 lines 10-12).

q) Data analysis. Due to the location of the 5-HTR2C gene on the X chromosome, the analyses should be performed separately considering males and females. Response: We agree and will perform a gender analysis (Page 8, lines 15).

r) Moreover, Authors should compare, as sensitivity analysis, suicide attempters with the same psychiatric diagnosis to healthy controls (not only suicide attempters in general) in order to reduce the influences of psychopathology on the observed differences. Checar mi respuesta porque no entiendo exactamente a que se refiere el revisor. We deeply appreciate the reviewer's suggestion. (Page 6, lines 9-10).

s) A meta-regression including age and ethnicity should be further performed. Response: We do not plan to perform a meta-regression as we do not have the right software available.

t) Finally, what about the possible presence of linkage disequilibrium among the investigated SNPs and haplotypic analyses? Response: We will calculate it (Page 8, lines 12-13).

u) DISCUSSION Authors should consider the suicidal behavior according to the stress-diathesis model, and discuss the results also on the basis of it. Response: We deeply appreciate the reviewer's suggestion. We have mentioned the Stress-diathesis model (Page 9, lines 1-4) and will also consider it when presenting the results.

v) Please, report strengths and limitations as well. Response: We have done so. (Page 9, lines 19-23 and Page 10 lines 1-2).

Minor remarks:

a) Abstract: I suggest to consider the 5-HTR2C as involved in suicidal behaviour and not as cause. Response: Yes, we have corrected it (Page 2, lines 5-8).

b) Discussion Authors wrote: "Those who attempt suicide and survive, apart of having serious injuries (such as broken bones, brain damage, or organ failure), very often develop depression and other mental health problems, as consequences". This sentence is not correct. Suicide attempters do not develop depression or other mental health problems; the latter may be worsen by suicide attempts. Response: Yes, we have deleted the paragraph

Authors wrote: "serotonin neurotransmission system has received the most consideration as a candidate gene in suicidal behavior studies due to its role in mood regulation and because its function is altered in suicide". Maybe Authors wanted to write "as a candidate system" and not gene.

Response: Thank you, we have changed it (Page 4, lines 13-14).

VERSION 2 – REVIEW

REVIEWER	Raffaella Calati University of Bologna
REVIEW RETURNED	30-Jul-2014

GENERAL COMMENTS	This manuscript does not add extremely relevant knowledge to scientific literature. Maybe that what Authors would like to perform in the future on its basis will be more useful.
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VERSION 2 – AUTHOR RESPONSE

Reviewer: 1 Reviewer Name: Raffaella Calati Institution and Country University of Bologna Please state any competing interests or state 'None declared': None declared

This manuscript does not add extremely relevant knowledge to scientific literature. Maybe that what Authors would like to perform in the future on its basis will be more useful.

Response: We highly appreciated this comment of reviewer #1. The protocol development in this study is in compliance with good practice in research and is the basis for more research in this topic.