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Association between HTR2C gene variants and suicidal behavior: protocol for systematic review and meta-analysis of genetic studies

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3 **Association between HTR2C gene variants and suicidal behavior: protocol**
4 **for systematic review and meta-analysis of genetic studies.**
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Abstract

Introduction: Suicide is a significant public health issue and one of the most common causes of death throughout the world. Suicidal behavior is a complex and multi-factorial problem; case-control studies have reported an association between the serotonin system and suicidal behavior; recently, the 5-HT2C gen has been suggested as a probable cause.

Methods and analysis: To have a more comprehensive understanding of the role of these gene, a meta-analysis of 5-HT2C with an up-to-date data will be performed. This meta-analysis will follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) criteria. Studies deemed for inclusion in the systematic review will scored for methodological quality using the Newcastle-Ottawa Assessment Scale (NOS). Also, to give more support to our analysis, we will take into consideration the GRADES scales procedures.

Ethics and dissemination: The study will describe the association between HTR2C gene and suicidal behavior. The results of this study will be disseminated by peer-reviewed publication and scientific presentations in Mexico and the world.

Protocol registration: PROSPERO CRD42014009213

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8 Strength and limitations of this study
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12 • This study could improve the understanding of the genetic component of
13 suicidal behavior.
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- 15 • This study protocol could show evidence of the role that the 5-HTR2C gene
16 plays in the suicidal behavior.
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- 19 • A small sample or the presence of high heterogeneity could be a limitation.
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Introduction

Suicide is best known as the act of taking one's own life voluntarily. But, the suicidal behavior includes a wide spectrum of actions that can be classified as follows: suicidal ideation (includes thoughts of ending life); attempted suicide (also called parasuicide), this has been defined as the act not resulting in death in which an individual deliberately hurts himself; and the completed suicide that is a self-destructive behavior that ends the life of the person who carries it out.¹⁻³ Suicide is a worldwide problem and it is increasing through time.⁴ 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. The suicide mortality in Mexico has increased slowly but steadily over the last 40 years, in this country, the suicide is one of the five leading causes of death, and the third between people between 15 and 24 years of age.^{5,6} There are studies that have tried to find and explain genetic causes of the disorder, because social factors alone can not explain completely the suicidal behavior.⁷

The receptor 5HT_{2C} has been studied in the association with suicidal behavior.⁴ This gene of serotonin receptor 2C lies on chromosome X, on position Xq24-q28 of the long arm. It has five introns and six exons and is, so far, the only G-protein binding receptor known to have the possibility of alternative splicing of mRNA and therefore of more protein isoforms.⁸ 5-HT_{2C} has been implicated in numerous brain functions, regulation of locomotion, sex, instinct and appetite to modulation of

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3 mood and anxiety among others.⁹ Since anxiety and mood disorders, as well as
4 impulsiveness, are important suicide risk factors, the changes in serotonin receptor
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8 2C could be linked to suicide.¹⁰ The HTR2C (5-HT2C gene) has a vast number of
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10 single nucleotide polymorphisms (SNPs). More of 1000 polymorphism have been
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12 identified along the gene (according to snpper.chip.org, October 2, 2013).¹¹ This
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14 gene has been associated with many diseases as schizophrenia,^{12 13} weigh gain in
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16 schizophrenia,¹⁴ bipolar disorder,^{15 16} alcoholism,¹⁷ and substance abuse,¹⁸
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19 However, the association between HTR2C and suicidal behavioral remains
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21 controversial, given that to date case-control studies include positive and negative
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23 findings.¹⁹⁻²³
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28 **Aims and objectives**

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31 Because of these controversial outcomes, the aims of the protocol study is to
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33 evaluate the relation of the HTR2C gene and the suicidal behavior with a meta-
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35 analysis. In order to have a better understanding of this association, we will study
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37 rs547536, rs2192372, rs4272555, rs6318 and rs2428707 polymorphisms of
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39 HTR2C gene in suicide attempters in Mexican population.
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47 **Methods/analysis**

48 49 50 51 52 53 **Meta-analysis**

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56 This meta-analysis will follow the Preferred Reporting Items for Systematic
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Reviews and Meta-Analyses (PRISMA) criteria.²⁴

Identification and selection of publications

The literature will be obtained from PubMed databases using the keywords: “HTR2C and suicidal behavior”, “HTR2C and suicide”. The dates of publication of the searched papers will be from 2003 to 2014, Figure 1. Publications selected will meet the following criteria: (1) to be published in peer-reviewed journals, (2) to be written in English, (3) to contain independent data, (4) to be case-control association studies in which the frequencies of three genotypes are clearly stated or could be calculated, and (5) the use of healthy individuals as controls.

Data extraction

Of each report the following data will be reviewed: authors, year of publication, region, number of cases and controls, gender, age, psychiatric diagnosis, ethnical origin, sample size and genotype and allele frequencies. The meta-analysis will be constructed taking into account the following categories: (a) exposed sick, (b) exposed not-sick, (c) not-exposed sick and (d) not- exposed not-sick. The “sick” term refers to subjects exhibiting suicidal behavior and the “exposed” term to the allele of risk.

Data analysis

For the meta-analysis analysis, we are going to use the EPIDAT 3.1 program (<http://dxsp.sergas.es>). This software is freely available for epidemiologic analysis

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3 of tabulated data. Data will be analyzed with the random-effects model following
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5 the reports in literature.²⁵⁻²⁷ Sample heterogeneity will be analyzed with the
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7 Dersimonian and Laird's Q test. The results of the Q test will be complemented
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9 with graphs to help visualize those studies favoring heterogeneity. The results of
10
11 the meta-analysis will be expressed as odds ratios (ORs). Pooled ORs will be
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13 calculated respectively for each of the models used, viz.: allelic (Example: A vs. C),
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15 additive (AA vs. CC), dominant (AA + AC vs. CC), and recessive (AA vs. AC +
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17 CC). To address the problem of publication bias, the Egger's test and funnel plots
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19 will be calculated with the EPIDAT 3.1 software. This plotting standardizes the
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21 effect of each study on the vertical axis and its correspondent precision on the
22
23 horizontal axis. Finally, a chi-squared (χ^2) analysis will be used to calculate the
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25 Hardy-Weinberg equilibrium to evaluate genotype distribution. Studies deemed for
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27 inclusion in the systematic review will be scored for methodological quality using
28
29 the Newcastle-Ottawa Assessment Scale (NOS).²⁸ A score of six will be
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31 considered as the cut-off point to distinguish higher from lower quality studies. Also
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33 to give more support to our analysis, we will take in consideration de GRADES
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35 scales procedures (<http://www.gradeworkinggroup.org>)
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44 Discussion

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47 Those who attempt suicide and survive, apart of having serious injuries (such as
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49 broken bones, brain damage, or organ failure), very often develop depression and
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51 other mental health problems, as consequences. It is well known that suicide not
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53 only affects the person who commits it, is also affects the health of the community;
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55 family and friends of people who commit suicide may experience shock, anger,
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3 guilt, and depression. The medical costs and lost wages associated with suicide
4 also take their toll on the community. It has been proposed the importance of the
5 analysis of the suicide; but before we can prevent suicide, we need to know how
6 big the problem is, where it is, and whom it affects.
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13 Besides the studies which reveal social factor that increase the possibility to
14 commit a suicide, there are others that support that it could exist a genetic link to
15 suicide; in these studies we observe that people who completed suicide or who
16 have had suicidal thoughts or behavior are more likely to have a family history of
17 suicide. Therefore we can suggest that more studies are necessary to understand
18 a possible genetic component that could contribute to suicidal tendencies²⁹. The
19 serotonin neurotransmission system has received the most consideration as a
20 candidate gene in suicidal behavior studies due to its role in mood regulation and
21 because its function is altered in suicide victims.³⁰ The most studied genes are the
22 tryptophan hydroxylase 1 and 2 (TPH-1 and TPH-2), serotonin transporter (5-HTT
23 or SLC6A4) and the serotonin receptors (5-HTR1A, 5-HTR1B, 5-HTR2A, 5-
24 HTR2C, etc).³¹ Being the last one the most recently studied in this area around the
25 world Figure 2. The gene 5-HTR2C is an excellent candidate, although its study
26 needs a detailed analysis because its chromosomal location.²⁹ Also, a systematic
27 review and meta-analysis could improve the understanding of a genetic component
28 in suicidal behavior. This study protocol could show evidence of the role that the 5-
29 HTR2C plays in the suicidal behavior in all population with an up-to-date meta-
30 analysis and systematic review with the existing publications.
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Ethics and dissemination: The study will describe the association between HTR2C gene and suicidal behavior. The results of this study will be disseminated by peer-reviewed publication and scientific presentations in Mexico and the world.

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Author's contribution:

TZCA and GCTB conceived the study, participated in the design and helped to draft the manuscript. GA, JRI and LNML helped to select the genes and polymorphisms in the study. TZCA, GCTB and A wrote the draft of the manuscript. All authors read, critically revised, and approved the final version of the manuscript.

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Competing interests statement:

The authors declare not to have any competing interests.

Figure legends

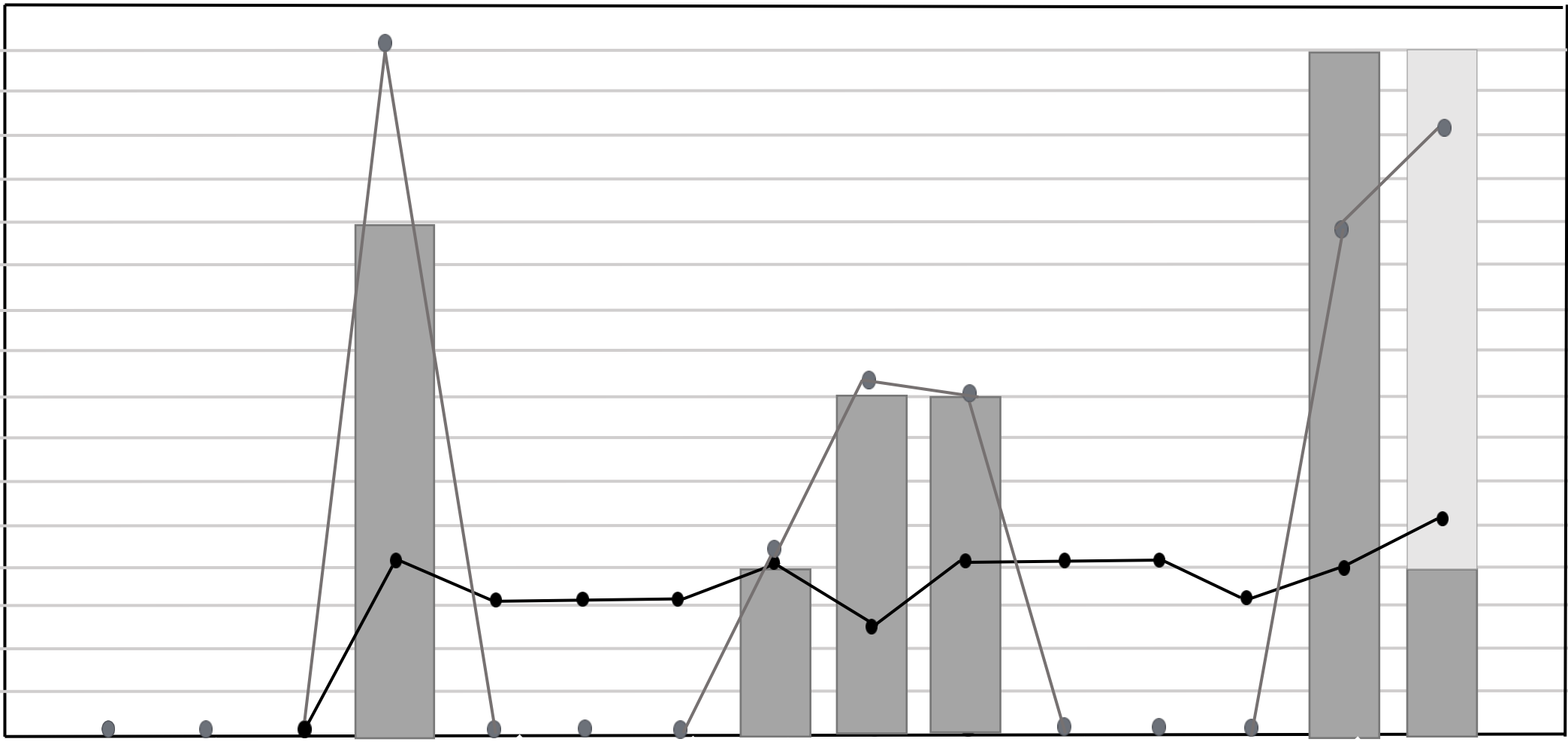
Figure 1

Representation of 5-HTR2C gene studies over time. (All data from: www.gopubmed.com)

Figure 2

World map of Genetic association studies of 5-HTR2C with suicidal behavior (All data from: www.gopubmed.com)

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Publications Publications (current year estimated) Relative Research Interest Relative Research Interest (smoothed)



World map of Genetic association studies of 5-HT_{2C} with suicidal behavior (All data from: www.gopubmed.com)

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2 **Abstract**

3 **Introduction:** Suicide is an important public health problem and one of the most
4 common causes of death throughout the world. Suicidal behavior is complex and
5 has multi-factorial causes; case-control studies have reported an association
6 between an alteration of the serotonin system and suicidal behavior; recently, the
7 5-HTRC2 serotonin receptor gene has been suggested to be involved in its
8 pathogenesis.

9 **Methods and analysis:** To evaluate the role of the 5-HTR2C gene in suicidal
10 behavior, we will perform a systematic review and a meta-analysis of world wide
11 reports that have investigated this association. This analysis will be reported
12 following the Preferred Reporting Items for Systematic Reviews and Meta-
13 Analyses (PRISMA) criteria. Studies deemed for inclusion in the systematic review
14 will be scored for methodological quality using the Newcastle-Ottawa Assessment
15 Scale (NOS). The inclusion criteria will be to present independent data, must be
16 case-control studies, and be published in journal peer-reviews. To generate more
17 accurate analyses, we will grade the reports using the GRADES scale procedures.

18 **Ethics and dissemination:** This study will describe the association between
19 HTR2C gene and suicidal behavior. The results will be disseminated by a peer-
20 reviewed publication and scientific presentations in Mexico and throughout the
21 world.

22 **Protocol registration:** PROSPERO CRD42014009213

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Strengths and limitations of the study

- The results could improve the understanding of the genetic component of suicidal behavior.
- This study could show evidence of an association between the 5-HTR2C gene and suicidal behavior.
- We will perform an analysis by gender that could determine if the 5-HTR2C gene affects female and male differently.
- A small amount of reports (and therefore a small sample size) is a possible limitation.
- High heterogeneity among the reports could be a limitation.

1

2 **Introduction**

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4 Suicide is the act of taking one's own life voluntarily and intentionally. Suicidal
5 behavior (SB) includes a wide spectrum of self-destructive actions that can be
6 classified as 1) suicidal ideation (thoughts of ending one's own life, including
7 planning a specific method to succeed), 2) attempted suicide or parasuicide (when
8 an individual deliberately hurts himself but the act does not result in death) and 3)
9 completed suicide (when the action ends the life of the person who carries it out).¹⁻³

10 Suicide is a worldwide problem and it is increasing through time.⁴⁻⁶ Social factors
11 alone can not fully explain the suicidal behavior,⁷⁻⁹ furthermore, some studies have
12 found that there is a potential genetic component in it.^{1 10}

13 The genetic studies that have tried to identify genes that predispose to suicidal
14 behavior, have focused on genes that codify proteins involved in the serotonergic
15 system, since this system interferes in controlling mood, emotions, aggression and
16 anxiety, this again could influence suicidal behavior.¹¹⁻¹³ Recently, the 5HTR2C
17 gene has been proposed to be related to suicidal behavior,^{10 14-17} due to its
18 association with other mental diseases such as schizophrenia,¹⁸⁻²⁰ bipolar
19 disorder,^{21 22} alcoholism,²³ and substance abuse.²⁴ Furthermore, the 5HTR2C gene
20 has been associated with anxiety, mood disorders and impulsiveness, and these
21 are important suicide risk factors.^{16 25}

22 The HTR2C (5-HTR2C gene) is a serotonin 2C receptor that lies on the X
23 chromosome, long arm, position Xq24-q28. It has five introns and six exons and so

1 far, is the only G-protein binding receptor known to alternative splicing the mRNA,
2 therefore to have more protein isoforms.²⁶ It has a vast number of single nucleotide
3 polymorphisms (SNPs). More of 1000 polymorphism have been identified along the
4 gene (according to snpper.chip.org, October 2, 2013).²⁷ To date, the association
5 between the HTR2C and suicidal behavioral remains controversial since the
6 reports include positive and negative findings.²⁸⁻³² The figure 1 shows the HTR2C
7 and suicide studies over time. Consequently, it is important to perform a deeper
8 study assessing the possible connection between the HTR2C gene and suicidal
9 behavior.

11 **Aims and objectives**

12 This study aims to systematically review and meta-analyze world wide reports that
13 have researched for an association between the serotonin 2C receptor gene (5-
14 HTR2C) and suicidal behavior. Particularly, it aims to focus on rs547536,
15 rs2192372, rs4272555, rs24287207 and rs6318 polymorphisms in order to detect
16 which ones act as a possible risk factor.

18 **Methods/analysis**

19 **Systematic Review and Meta-analysis**

20 This meta-analysis will be reported following the Preferred Reporting Items for
21 Systematic Reviews and Meta-Analyses (PRISMA) criteria.³³

1 Identification and selection of publications

2 For both, the systematic review and the meta-analysis, the literature will be
3 searched from three databases: PubMed, Web of Science and EBSCO using the
4 keywords: "HTR2C and suicidal behavior", "HTR2C and suicide". These words will
5 be combined to retrieve the summaries.

6 Publications selected will meet the following criteria: (1) to be published in peer-
7 reviewed journals, (2) to contain independent data, (3) to be case-control
8 association studies in which the frequencies of three genotypes are clearly stated
9 or could be calculated, (4) the use of healthy individuals or psychiatric patients
10 without suicidal behavior as controls; (5) to include one or more of the three forms
11 of suicidal behavior (suicidal ideation, attempted suicide or completed suicide); and
12 (6) to be written in English. The procedure planned is exemplified on figure 2.

13 To determine inclusion, two researchers (González-Castro and Juárez-Rojop) will
14 work independently screening each of the titles, abstracts and full texts. When the
15 researchers disagree a third researcher (Tovilla-Zárate) will be consulted.

17 Data extraction

18 For the systematic review, the following data will be searched and analyzed:
19 authors, year of publication, region, sample size, number of cases and controls,
20 gender, age, psychiatric diagnosis, ethnical origin and finally, genotype and allele
21 frequencies. The SNPs in study will be rs547536, rs2192372, rs4272555,

1 rs24287207 and rs6318.

2 The meta-analysis will be constructed following four categories: (a) exposed sick,
3 (b) exposed not-sick, (c) not-exposed sick and (d) not- exposed not-sick. The “sick”
4 term refers to subjects exhibiting suicidal behavior and the “exposed” term to the
5 allele of risk.

6 The data extraction will be performed by González-Castro, Juárez-Rojop, Genis-
7 Mendoza and López-Nárvaez.

8 **Data analysis**

9 The systematic review would be presented on tables comparing quality
10 measurements and the data previously mentioned. Studies deemed for inclusion
11 will be scored for methodological quality using the Newcastle-Ottawa Assessment
12 Scale (NOS)³⁴. A score of six will be considered as the cut-off point to distinguish
13 higher from lower quality studies. To give more support to our analysis, we will
14 consider the GRADES scale procedures (<http://www.gradeworkinggroup.org>).

15 For the meta-analysis we will use the EPIDAT 3.1 program (Software for
16 Epidemiologic Analysis of Tabulated Data. Version 3.1. Pan American Health
17 Organization; <http://dxsp.sergas.es>); this software is freely available for
18 epidemiologic analysis of tabulated data. Data will be analyzed using the random-
19 effects model following the reports in literature³⁵⁻³⁷. The sample heterogeneity will
20 be measured with the Dersimonian and Laird’s Q test; we will also use graphs to
21 help visualize those studies favoring heterogeneity. The results of the meta-
22 analysis will be expressed as odds ratios (ORs). Pooled ORs will be calculated for

1 each model used, viz.: allelic (Example: A vs. C), additive (AA vs. CC), dominant
2 (AA + AC vs. CC), and recessive (AA vs. AC + CC). Since the HTR2C gene is
3 located on the X chromosome, an analysis by gender will be performed. To
4 address the publication bias, the Egger's test and funnel plots will be calculated
5 with the EPIDAT 3.1 software. This plotting will standardize the effect of each study
6 on the vertical axis and its correspondent precision on the horizontal axis.
7 Sensibility analysis will be performed. The influence of each study in the global
8 estimation will be analyzed and graphed. The direction of the effect will be
9 analyzed. To evaluate genotype distribution and calculate the Hardy-Weinberg
10 equilibrium, we will use a chi-squared (χ^2) analysis. Regarding the presence of
11 Hardy-Weinberg equilibrium, we will use the Pearson's chi-squared test to assess
12 goodness of fit. Finally, the Haploview 4.2³⁸ will be used to calculate the linkage
13 disequilibrium (LD) of the markers.

14 The analyzed of dates will be performed by Gonzalez-Castro and Tovilla-Zarate-
15 Differences in the interpretation could be resolved by López-Narvaez.

16

17 Discussion

18 Suicide is a public health problem that affects entire communities and not only the
19 suicidal person. The medical costs and lost wages associated with suicide also
20 take their toll on the community. Therefore, it is crucial to better understand the
21 etiology of suicidal behavior, as well as knowing how big the problem is and whom
22 it affects.

1 Literature suggests that there could be a genetic factor linked to suicide. Some
2 studies have proposed a hypothetical explanation using the Stress-Diathesis Model
3 and have observed that people who have had suicidal behavior are more likely to
4 have a family history of suicide.¹⁶ Therefore we can suggest that a genetic
5 component could contribute to suicidal tendencies.¹⁶ The serotonin
6 neurotransmission system has received the most consideration as candidate
7 genes in suicidal behavior, since the serotonin system regulates mood,
8 impulsiveness and anxiety which are important suicide risk factors. Furthermore,
9 the serotonergic function is clearly altered in suicide victims.¹⁰ Among the most
10 studied genes, are the ones that codify to the tryptophan hydroxylase 1 and 2
11 (TPH-1 and TPH-2), serotonin transporter (5-HTT or SLC6A4) and the serotonin
12 receptors (5-HTR1A, 5-HTR1B, 5-HTR2A, 5-HTR2C, etc)³⁹. Being the last one the
13 most recently suggested around the world to be associated with suicidal behavior
14 Figure 3. The gene 5-HTR2C needs a deeper analysis Performing a systematic
15 review and a meta-analysis, this protocol could improve the understanding of the
16 genetic component in suicidal behavior; regarding the data analysis, we will adopt
17 the random effects model, because it accounts for additional sources of inter-study
18 variation when heterogeneity exists and has a more reliable outcome.

19 This meta-analysis could present some limitations. First, the association between
20 HTR2C and suicidal behavior is not widely studied; therefore, we might not find a
21 sufficient amount of reports to analyze; furthermore, we are focusing on five
22 polymorphisms and perhaps there are not enough reports of each one. Secondly,
23 the reports will be limited to English language so the number of studies might be

1 too small. High heterogeneity due to differences in age, ethnicity, gender,
2 education, wealth, etc. These limitations will decrease the statistical power of the
3 study o reliability of our results.

4 Conversely, it would be the first meta-analysis associating between five SNPs of
5 HTR2C and suicidal behavior with allelic, additive, dominant, and recessive
6 models. In addition, this study protocol could demonstrate the link between the 5-
7 HTR2C in the suicidal behavior of the general population.

8 Performing an analysis by gender is a strong point; in theory, due to its
9 chromosomal location (X chromosome), this gene must affect females and males
10 differently. The results could help to determine if the HTR2C gene is a risk factor in
11 developing suicidal behavior. Indirectly, our study could help to understand the
12 etiology of suicidal behavior.

13
14 **Ethics and dissemination:** This study will describe the association between
15 HTR2C gene and suicidal behavior. The results of this study will be disseminated
16 by peer-reviewed publication and scientific presentations in Mexico and throughout
17 the world.

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67 **2 Author's contribution:**
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10 3 TZCA and GCTB conceived the study, participated in the design and helped to
11 4 draft the manuscript. GA, JRI and LNML helped on the selection of genes and
12 5 polymorphisms to study. TZCA, GCTB and A wrote the manuscript. All authors
13 6 read, critically revised and approved the final version of the manuscript.
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2021 8 **Funding statement:**
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28 10 **Competing interests statement:**
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31 11 The authors declare not to have any competing interests.
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5 2 **Figure legends**6
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9 3 Figure 110
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12 4 Representation of 5-HTR2C gene studies over time. (All data from:
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14 www.gopubmed.com)
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18 6 **Figure 2.**19
20 7 Flow-chart for the search strategy and the inclusion/exclusion criteria to be used in
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22 8 the meta-analysis and systematic review.
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28 10 Figure 329
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31 11 World map of Genetic association studies of 5-HTR2C with suicidal behavior (All
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3 **1 Association between HTR2C gene variants and suicidal behavior: protocol**
4 **2 for systematic review and meta-analysis of genetic studies.**
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9 4 González-Castro Thelma Beatriz¹, Juárez-Rojop Isela¹, Genis Alma², López-
10 5 Narváez María Lilia³ Tovilla-Zárate Carlos Alfonso⁴ *

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2 **Abstract**

3 **Introduction:** Suicide is an important public health problem and one of the most
4 common causes of death throughout the world. Suicidal behavior is complex and
5 has multi-factorial causes; case-control studies have reported an association
6 between an alteration of the serotonin system and suicidal behavior; recently, the
7 5-HTRC2 serotonin receptor gene has been suggested to be involved in its
8 pathogenesis.

9 **Methods and analysis:** To evaluate the role of the 5-HTR2C gene in suicidal
10 behavior, we will perform a systematic review and a meta-analysis of world wide
11 reports that have investigated this association. This analysis will be reported
12 following the Preferred Reporting Items for Systematic Reviews and Meta-
13 Analyses (PRISMA) criteria. Studies deemed for inclusion in the systematic review
14 will be scored for methodological quality using the Newcastle-Ottawa Assessment
15 Scale (NOS). The inclusion criteria will be to present independent data, must be
16 case-control studies, and be published in journal peer-reviews. To generate more
17 accurate analyses, we will grade the reports using the GRADES scale procedures.

18 **Ethics and dissemination:** This study will describe the association between
19 HTR2C gene and suicidal behavior. The results will be disseminated by a peer-
20 reviewed publication and scientific presentations in Mexico and throughout the
21 world.

22 **Protocol registration:** PROSPERO CRD42014009213

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8 3 Strengths and limitations of the study
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- 12 • The results could improve the understanding of the genetic component of
13 suicidal behavior.
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 - 15 • This study could show evidence of an association between the 5-HTR2C
16 gene and suicidal behavior.
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 - 18 • We will perform an analysis by gender that could determine if the 5-HTR2C
19 gene affects female and male differently.
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 - 21 • A small amount of reports (and therefore a small sample size) is a possible
22 limitation.
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 - 24 • High heterogeneity among the reports could be a limitation.
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67 2 **Introduction**8
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10 4 Suicide is the act of taking one's own life voluntarily and intentionally. Suicidal
11 behavior (SB) includes a wide spectrum of self-destructive actions that can be
12 classified as 1) suicidal ideation (thoughts of ending one's own life, including
13 planning a specific method to succeed), 2) attempted suicide or parasuicide (when
14 an individual deliberately hurts himself but the act does not result in death) and 3)
15 completed suicide (when the action ends the life of the person who carries it out).¹⁻³

16 6 Suicide is a worldwide problem and it is increasing through time.⁴⁻⁶ Social factors
17 alone can not fully explain the suicidal behavior,⁷⁻⁹ furthermore, some studies have
18 found that there is a potential genetic component in it.^{1 10}

19 8 The genetic studies that have tried to identify genes that predispose to suicidal
20 behavior, have focused on genes that codify proteins involved in the serotonergic
21 system, since this system interferes in controlling mood, emotions, aggression and
22 anxiety, this again could influence suicidal behavior.¹¹⁻¹³ Recently, the 5HTR2C
23 gene has been proposed to be related to suicidal behavior,^{10 14-17} due to its
24 association with other mental diseases such as schizophrenia,¹⁸⁻²⁰ bipolar
25 disorder,^{21 22} alcoholism,²³ and substance abuse.²⁴ Furthermore, the 5HTR2C gene
26 has been associated with anxiety, mood disorders and impulsiveness, and these
27 are important suicide risk factors.^{16 25}

28 10 The HTR2C (5-HTR2C gene) is a serotonin 2C receptor that lies on the X
29 chromosome, long arm, position Xq24-q28. It has five introns and six exons and so

1 far, is the only G-protein binding receptor known to alternative splicing the mRNA,
2 therefore to have more protein isoforms.²⁶ It has a vast number of single nucleotide
3 polymorphisms (SNPs). More of 1000 polymorphism have been identified along the
4 gene (according to snpper.chip.org, October 2, 2013).²⁷ To date, the association
5 between the HTR2C and suicidal behavioral remains controversial since the
6 reports include positive and negative findings.²⁸⁻³² The figure 1 shows the HTR2C
7 and suicide studies over time. Consequently, it is important to perform a deeper
8 study assessing the possible connection between the HTR2C gene and suicidal
9 behavior.

11 Aims and objectives

12 This study aims to systematically review and meta-analyze world wide reports that
13 have researched for an association between the serotonin 2C receptor gene (5-
14 HTR2C) and suicidal behavior. Particularly, it aims to focus on rs547536,
15 rs2192372, rs4272555, rs24287207 and rs6318 polymorphisms in order to detect
16 which ones act as a possible risk factor.

18 Methods/analysis

19 Systematic Review and Meta-analysis

20 This meta-analysis will be reported following the Preferred Reporting Items for
21 Systematic Reviews and Meta-Analyses (PRISMA) criteria.³³

1 Identification and selection of publications

2 For both, the systematic review and the meta-analysis, the literature will be
3 searched from three databases: PubMed, Web of Science and EBSCO using the
4 keywords: "HTR2C and suicidal behavior", "HTR2C and suicide". These words will
5 be combined to retrieve the summaries.

6 Publications selected will meet the following criteria: (1) to be published in peer-
7 reviewed journals, (2) to contain independent data, (3) to be case-control
8 association studies in which the frequencies of three genotypes are clearly stated
9 or could be calculated, (4) the use of healthy individuals or psychiatric patients
10 without suicidal behavior as controls; (5) to include one or more of the three forms
11 of suicidal behavior (suicidal ideation, attempted suicide or completed suicide); and
12 (6) to be written in English. The procedure planned is exemplified on figure 2.

13 To determine inclusion, two researchers (González-Castro and Juárez-Rojop) will
14 work independently screening each of the titles, abstracts and full texts. When the
15 researchers disagree a third researcher (Tovilla-Zárate) will be consulted.

17 Data extraction

18 For the systematic review, the following data will be searched and analyzed:
19 authors, year of publication, region, sample size, number of cases and controls,
20 gender, age, psychiatric diagnosis, ethnical origin and finally, genotype and allele
21 frequencies. The SNPs in study will be rs547536, rs2192372, rs4272555,

1 rs24287207 and rs6318.

2 The meta-analysis will be constructed following four categories: (a) exposed sick,
3 (b) exposed not-sick, (c) not-exposed sick and (d) not- exposed not-sick. The “sick”
4 term refers to subjects exhibiting suicidal behavior and the “exposed” term to the
5 allele of risk.

6 The data extraction will be performed by González-Castro, Juárez-Rojop, Genis-
7 Mendoza and López-Nárvaez.

8 **Data analysis**

9 The systematic review would be presented on tables comparing quality
10 measurements and the data previously mentioned. Studies deemed for inclusion
11 will be scored for methodological quality using the Newcastle-Ottawa Assessment
12 Scale (NOS)³⁴. A score of six will be considered as the cut-off point to distinguish
13 higher from lower quality studies. To give more support to our analysis, we will
14 consider the GRADES scale procedures (<http://www.gradeworkinggroup.org>).

15 For the meta-analysis we will use the EPIDAT 3.1 program (Software for
16 Epidemiologic Analysis of Tabulated Data. Version 3.1. Pan American Health
17 Organization; <http://dxsp.sergas.es>); this software is freely available for
18 epidemiologic analysis of tabulated data. Data will be analyzed using the random-
19 effects model following the reports in literature³⁵⁻³⁷. The sample heterogeneity will
20 be measured with the Dersimonian and Laird’s Q test; we will also use graphs to
21 help visualize those studies favoring heterogeneity. The results of the meta-
22 analysis will be expressed as odds ratios (ORs). Pooled ORs will be calculated for

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3 1 each model used, viz.: allelic (Example: A vs. C), additive (AA vs. CC), dominant
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5 2 (AA + AC vs. CC), and recessive (AA vs. AC + CC). Since the HTR2C gene is
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7
8 3 located on the X chromosome, an analysis by gender will be performed. To
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10 4 address the publication bias, the Egger's test and funnel plots will be calculated
11
12 5 with the EPIDAT 3.1 software. This plotting will standardize the effect of each study
13
14 6 on the vertical axis and its correspondent precision on the horizontal axis.
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16
17 7 Sensibility analysis will be performed. The influence of each study in the global
18
19 8 estimation will be analyzed and graphed. The direction of the effect will be
20
21 9 analyzed. To evaluate genotype distribution and calculate the Hardy-Weinberg
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23 10 equilibrium, we will use a chi-squared (χ^2) analysis. Regarding the presence of
24
25 11 Hardy-Weinberg equilibrium, we will use the Pearson's chi-squared test to assess
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27 12 goodness of fit. Finally, the Haploview 4.2³⁸ will be used to calculate the linkage
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29 13 disequilibrium (LD) of the markers.
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35 14 The analyzed of dates will be performed by Gonzalez-Castro and Tovilla-Zarate-
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37 15 Differences in the interpretation could be resolved by López-Narvaez.
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44 17 Discussion

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47 18 Suicide is a public health problem that affects entire communities and not only the
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49 19 suicidal person. The medical costs and lost wages associated with suicide also
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51 20 take their toll on the community. Therefore, it is crucial to better understand the
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53 21 etiology of suicidal behavior, as well as knowing how big the problem is and whom
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55 22 it affects.
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1 Literature suggests that there could be a genetic factor linked to suicide. Some
2 studies have proposed a hypothetical explanation using the Stress-Diathesis Model
3 and have observed that people who have had suicidal behavior are more likely to
4 have a family history of suicide.¹⁶ Therefore we can suggest that a genetic
5 component could contribute to suicidal tendencies.¹⁶ The serotonin
6 neurotransmission system has received the most consideration as candidate
7 genes in suicidal behavior, since the serotonin system regulates mood,
8 impulsiveness and anxiety which are important suicide risk factors. Furthermore,
9 the serotonergic function is clearly altered in suicide victims.¹⁰ Among the most
10 studied genes, are the ones that codify to the tryptophan hydroxylase 1 and 2
11 (TPH-1 and TPH-2), serotonin transporter (5-HTT or SLC6A4) and the serotonin
12 receptors (5-HTR1A, 5-HTR1B, 5-HTR2A, 5-HTR2C, etc)³⁹. Being the last one the
13 most recently suggested around the world to be associated with suicidal behavior
14 Figure 3. The gene 5-HTR2C needs a deeper analysis Performing a systematic
15 review and a meta-analysis, this protocol could improve the understanding of the
16 genetic component in suicidal behavior; regarding the data analysis, we will adopt
17 the random effects model, because it accounts for additional sources of inter-study
18 variation when heterogeneity exists and has a more reliable outcome.

19 This meta-analysis could present some limitations. First, the association between
20 HTR2C and suicidal behavior is not widely studied; therefore, we might not find a
21 sufficient amount of reports to analyze; furthermore, we are focusing on five
22 polymorphisms and perhaps there are not enough reports of each one. Secondly,
23 the reports will be limited to English language so the number of studies might be

1 too small. High heterogeneity due to differences in age, ethnicity, gender,
2 education, wealth, etc. These limitations will decrease the statistical power of the
3 study o reliability of our results.

4 Conversely, it would be the first meta-analysis associating between five SNPs of
5 HTR2C and suicidal behavior with allelic, additive, dominant, and recessive
6 models. In addition, this study protocol could demonstrate the link between the 5-
7 HTR2C in the suicidal behavior of the general population.

8 Performing an analysis by gender is a strong point; in theory, due to its
9 chromosomal location (X chromosome), this gene must affect females and males
10 differently. The results could help to determine if the HTR2C gene is a risk factor in
11 developing suicidal behavior. Indirectly, our study could help to understand the
12 etiology of suicidal behavior.

13
14 **Ethics and dissemination:** This study will describe the association between
15 HTR2C gene and suicidal behavior. The results of this study will be disseminated
16 by peer-reviewed publication and scientific presentations in Mexico and throughout
17 the world.

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2 Author's contribution:

3 TZCA and GCTB conceived the study, participated in the design and helped to
4 draft the manuscript. GA, JRI and LNML helped on the selection of genes and
5 polymorphisms to study. TZCA, GCTB and A wrote the manuscript. All authors
6 read, critically revised and approved the final version of the manuscript.

7

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10 Competing interests statement:

11 The authors declare not to have any competing interests.

12

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2 **Figure legends**

3 Figure 1

4 Representation of 5-HTR2C gene studies over time. (All data from:
5 www.gopubmed.com)

6 **Figure 2.**

7 Flow-chart for the search strategy and the inclusion/exclusion criteria to be used in
8 the meta-analysis and systematic review.

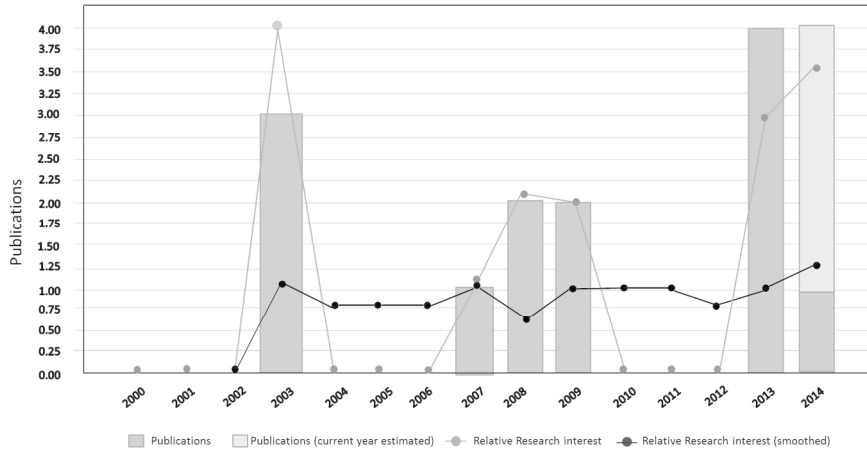
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10 Figure 3

11 World map of Genetic association studies of 5-HTR2C with suicidal behavior (All
12 data from: www.gopubmed.com)

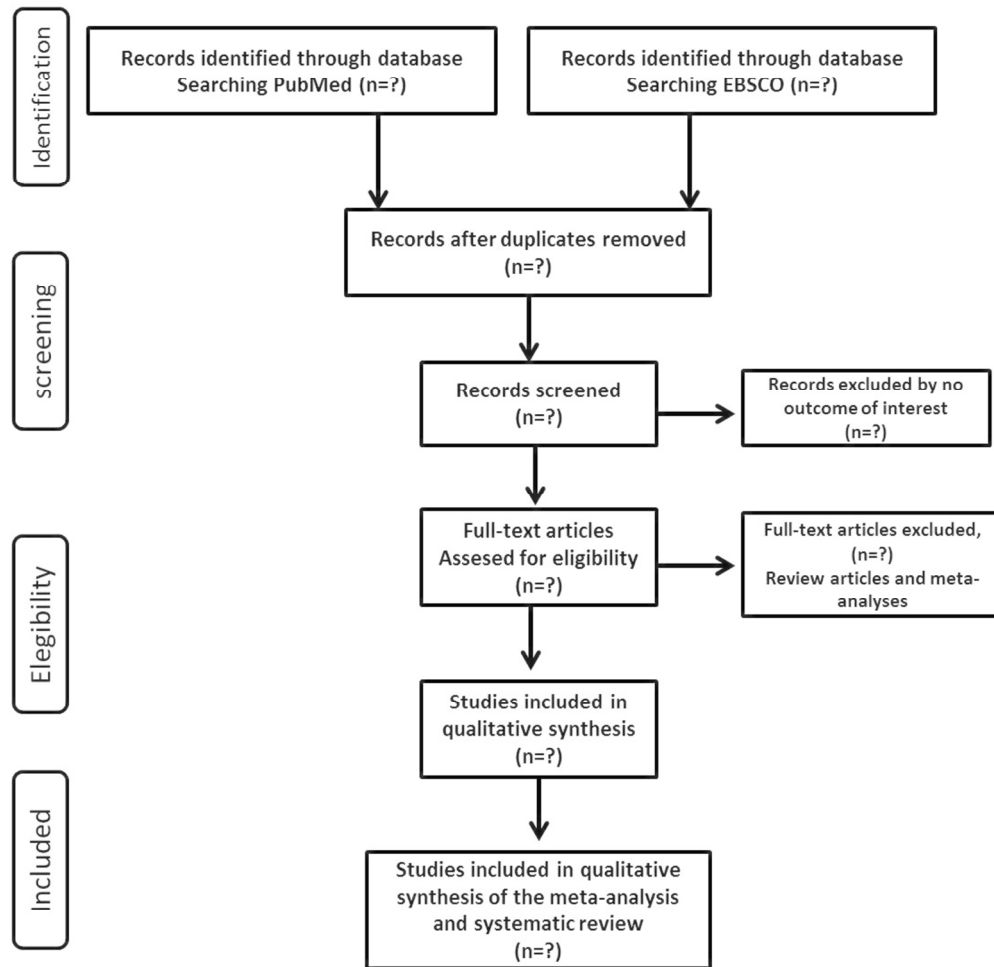
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Distribution of association studies on the 5-HTR2C gene and suicidal behaviour published in PubMed by year (figure adapted from the results of GoPubMed®).
202x110mm (300 x 300 DPI)

review only



Flow-chart for the search strategy and the inclusion/exclusion criteria used in the meta-analysis and systematic review.
93x97mm (300 x 300 DPI)



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World map of genetic association studies of the 5-HTT2C gene with suicidal behaviour (figure adapted from the results of GoPubMed®).

er review only

BMJ Open

Association between HTR2C gene variants and suicidal behaviour: A protocol for the systematic review and meta-analysis of genetic studies

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2014-005423.R2
Article Type:	Protocol
Date Submitted by the Author:	19-Aug-2014
Complete List of Authors:	González-Castro, Thelma; Universidad Juárez Autónoma de Tabasco, División Académica de Ciencias de la Salud Juárez-Rojop, Isela; Universidad Juárez Autónoma de Tabasco, División Académica de Ciencias de la Salud Genis-Mendoza, Alma; Instituto de Medicina Genómica (INMEGEN), Servicios de Atención Psiquiátrica (SAP), Secretaría de Salud López-Narváez, María; Hospital General de Yajalon, Secretaría de Salud Tovilla-Zárate, Carlos; Universidad Juárez Autónoma de Tabasco, División Multidisciplinaria de Comalcalco, División Académica Multidisciplinaria de Comalcalco
Primary Subject Heading:	Mental health
Secondary Subject Heading:	Mental health
Keywords:	PSYCHIATRY, Suicide & self-harm < PSYCHIATRY, STATISTICS & RESEARCH METHODS

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Manuscripts

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6 3 **Association between HTR2C gene variants and suicidal behaviour: A**
7 4 **protocol for the systematic review and meta-analysis of genetic studies**
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Abstract

Introduction: Suicide is an important public health problem and one of the most common causes of death throughout the world. Suicidal behaviour is complex, and its causes are multi-factorial. Case-control studies have reported an association between an alteration of the serotonin system and suicidal behaviour. Recently, it has been suggested that the 5-HT_{2C} serotonin receptor gene is involved in the pathogenesis of suicidal behaviour.

Methods and analysis: To evaluate the role of the 5-HT_{2C} gene in suicidal behaviour, we will perform a systematic review and a meta-analysis of worldwide reports that have investigated the association between the serotonin system and suicidal behaviour. This analysis will be reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) criteria. Studies deemed for inclusion in the systematic review will be scored for methodological quality using the Newcastle-Ottawa Assessment Scale (NOS). The inclusion criteria will be to present independent data, to be case-control studies, and to be published in journal peer-reviews. To generate more accurate analyses, we will grade the reports using the GRADES scale procedures.

Ethics and dissemination: This study will describe the association between the HTR_{2C} gene and suicidal behaviour. The results will be reported in a peer-reviewed publication and in scientific presentations in Mexico and throughout the world.

Protocol registration: PROSPERO CRD42014009213

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Strengths and limitations of the study

- The results could improve the understanding of the genetic component of suicidal behaviour.
- This study could show evidence of an association between the 5-HTR2C gene and suicidal behaviour.
- We will perform an analysis by gender that could determine if the 5-HTR2C gene affects females and males differently.
- A small number of reports (and therefore a small sample size) is a possible limitation.
- High heterogeneity among the reports could be a limitation.

1

2 Introduction

3

4 Suicide is the act of taking one's own life voluntarily and intentionally. Suicidal
5 behaviour (SB) includes a wide spectrum of self-destructive actions that can be
6 classified as: 1) suicidal ideation (thoughts of ending one's own life, including
7 planning a specific method to succeed), 2) attempted suicide or parasuicide (when
8 an individual deliberately hurts himself but the act does not result in death) and 3)
9 completed suicide (when the action ends the life of the person who carries it out).¹⁻³

10 Suicide is a worldwide problem, and its prevalence has been increasing over
11 time.⁴⁻⁶ Social factors alone cannot fully explain suicidal behaviour.⁷⁻⁹ Furthermore,
12 some studies have found that there is a potential genetic component linked to
13 suicidal behaviour.^{1 10}

14 The genetic studies that have tried to identify genes that predispose certain
15 individuals to suicidal behaviour have focused on genes that codify the proteins
16 involved in the serotonergic system because this system interferes with the
17 control of mood, emotions, aggression and anxiety, all of which could influence
18 suicidal behaviour.¹¹⁻¹³ Recently, it has been proposed that the 5-HTR2C gene is
19 related to suicidal behaviour^{10 14-17} due to its association with other mental diseases
20 such as schizophrenia,¹⁸⁻²⁰ bipolar disorder,^{21 22} alcoholism,²³ and substance
21 abuse.²⁴ Furthermore, the 5-HTR2C gene has been associated with anxiety, mood
22 disorders and impulsiveness, and these are important suicide risk factors.^{16 25}

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3 1 HTR2C (the 5-HTR2C gene) is a serotonin 2C receptor that lies on the X
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5 2 chromosome on the long arm at position Xq24-q28. HTR2C has five introns and six
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8 3 exons, and so far, it is the only G-protein binding receptor known to undergo
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10 4 alternative splicing of the mRNA, resulting in more protein isoforms.²⁶ HTR2C has
11
12 5 a vast number of single nucleotide polymorphisms (SNPs). More than 1000
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14 6 polymorphism have been identified along the gene (according to snpper.chip.org,
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16 7 October 2, 2013).²⁷ To date, the association between HTR2C and suicidal
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18 8 behaviour remains controversial because the reports include both positive and
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20 9 negative findings.²⁸⁻³² Figure 1 shows the distribution of HTR2C and suicide
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22 10 studies over time. Consequently, it is important to perform a deeper study
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24 11 assessing the possible connection between the 5-HTR2C gene and suicidal
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26 12 behaviour.
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35 14 **Aims and objectives**

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38 15 This study aims to systematically review and perform a meta-analysis on the
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40 16 worldwide reports that have investigated an association between the serotonin 2C
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42 17 receptor gene (5-HTR2C) and suicidal behaviour. Particularly, it aims to focus on
43
44 18 rs547536, rs2192372, rs4272555, rs24287207 and rs6318 polymorphisms to
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46 19 detect which ones act as possible risk factors.
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53 21 **Methods/analysis**

54 55 56 22 **Systematic Review and Meta-analysis** 57 58 59 60

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3 1 This meta-analysis will be reported following the Preferred Reporting Items for
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5 2 Systematic Reviews and Meta-Analyses (PRISMA) criteria.³³
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8 9 **Identification and selection of publications**

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12 4 For both the systematic review and the meta-analysis, the literature will be
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14 5 searched from three databases—PubMed, Web of Science and EBSCO—using
15
16 6 the following keywords: “HTR2C and suicidal behaviour” and “HTR2C and suicide”.
17
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19 7 These words will be combined to retrieve the summaries.
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23 8 The selected publications will meet the following criteria: (1) to be published in
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25 9 peer-reviewed journals, (2) to contain independent data, (3) to be case-control
26
27 10 association studies in which the frequencies of the three genotypes are clearly
28
29 11 stated or could be calculated, (4) to use healthy individuals or psychiatric patients
30
31 12 without suicidal behaviour as controls, (5) to include one or more of the three forms
32
33 13 of suicidal behaviour (suicidal ideation, attempted suicide or completed suicide),
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35 14 and (6) to be written in English. The planned procedure is illustrated in Figure 2.
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39
40 15 To determine inclusion, two researchers (González-Castro and Juárez-Rojop) will
41
42 16 work independently to screen each of the titles, abstracts and full texts. When the
43
44 17 researchers disagree a third researcher (Tovilla-Zárate) will be consulted.
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50 51 **Data extraction**

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54 20 For the systematic review, the following data will be searched and analysed:
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56 21 authors, year of publication, region, sample size, number of cases and controls,
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1 gender, age, psychiatric diagnosis, ethnical origin and finally, genotype and allele
2 frequencies. The SNPs in the study will be rs547536, rs2192372, rs4272555,
3 rs24287207 and rs6318.

4 The meta-analysis will be constructed following four categories: (a) exposed sick,
5 (b) exposed not-sick, (c) not-exposed sick and (d) not- exposed not-sick. The term
6 “sick” refers to subjects exhibiting suicidal behaviour and the term “exposed” refers
7 to the allele of risk.

8 The data extraction will be performed by González-Castro, Juárez-Rojop, Genis-
9 Mendoza and López-Nárvaez.

10 **Data analysis**

11 The systematic review will be presented in tables comparing quality measurements
12 and the data previously mentioned. Studies deemed for inclusion will be scored for
13 methodological quality using the Newcastle-Ottawa Assessment Scale (NOS).³⁴ A
14 score of six will be considered the cut-off point to distinguish higher from lower
15 quality studies. To give more support to our analysis, we will consider the GRADES
16 scale procedures (<http://www.gradeworkinggroup.org>).

17 For the meta-analysis, we will use the EPIDAT 3.1 program (Software for
18 Epidemiologic Analysis of Tabulated Data, Version 3.1., Pan American Health
19 Organization; <http://dxsp.sergas.es>); this software is freely available for the
20 epidemiologic analysis of tabulated data. Data will be analysed using the random-
21 effects model following the reports in the literature.³⁵⁻³⁷ The sample heterogeneity
22 will be measured using the Dersimonian and Laird’s Q test; we will also use graphs

1 to help visualise those studies favouring heterogeneity. The results of the meta-
2 analysis will be expressed as odds ratios (ORs). Pooled ORs will be calculated for
3 each model used: viz.: allelic (Example: A vs. C), additive (AA vs. CC), dominant
4 (AA + AC vs. CC), and recessive (AA vs. AC + CC). Because the HTR2C is
5 located on the X chromosome, an analysis by gender will be performed. To
6 address the publication bias, the Egger's test and funnel plots will be calculated
7 with the EPIDAT 3.1 software. This plotting will standardize the effect of each study
8 on the vertical axis and its correspondent precision on the horizontal axis.
9 Sensibility analysis will be performed. The influence of each study in the global
10 estimation will be analysed and graphed. The direction of the effect will be
11 analysed. To evaluate genotype distribution and calculate the Hardy-Weinberg
12 equilibrium, we will use a chi-squared (χ^2) analysis. Regarding the presence of
13 Hardy-Weinberg equilibrium, we will use the Pearson's chi-squared test to assess
14 goodness of fit. Finally, the Haploview 4.2³⁸ will be used to calculate the linkage
15 disequilibrium (LD) of the markers.

16 The analysed of dates will be performed by Gonzalez-Castro and Tovilla-Zarate-
17 Any differences in the interpretation will be resolved by López-Narvaez.

19 Discussion

20 Suicide is a public health problem that affects entire communities and not just the
21 suicidal person. The medical costs and lost wages associated with suicide also
22 take their toll on the community. Therefore, it is crucial to better understand the

1 aetiology of suicidal behaviour as well as define how large the problem is and
2 whom it affects.

3 The literature suggests that there could be a genetic factor linked to suicide. Some
4 studies have proposed a hypothetical explanation using the Stress-Diathesis Model
5 and have observed that people who exhibit suicidal behaviour are more likely to
6 have a family history of suicide.¹⁶ Therefore, this finding suggests that a genetic
7 component could contribute to suicidal tendencies.¹⁶ The serotonin
8 neurotransmission system has received the most consideration as the candidate
9 genes linked to suicidal behaviour because the serotonin system regulates mood,
10 impulsiveness and anxiety, which are important suicide risk factors. Furthermore,
11 the serotonergic function is clearly altered in suicide victims.¹⁰ Among the most
12 studied genes are the ones that codify tryptophan hydroxylase 1 and 2 (TPH-1 and
13 TPH-2), the serotonin transporter (5-HTT or SLC6A4) and the serotonin receptors
14 (5-HTR1A, 5-HTR1B, 5-HTR2A, 5-HTR2C, etc.).³⁹ Figure 3 summarizes the
15 worldwide studies linking suicidal behaviour with HTR2C, the gene most recently
16 highlighted in the literature in association with suicidal behaviour. The 5-HTR2C
17 gene requires a deeper analysis; therefore, by performing a systematic review and
18 a meta-analysis, this protocol could improve the understanding of the genetic
19 component in suicidal behaviour. Regarding the data analysis, we will adopt the
20 random effects model because it accounts for additional sources of inter-study
21 variation when heterogeneity exists and thus has a more reliable outcome.

22 This meta-analysis could present some limitations. First, the association between
23 HTR2C and suicidal behaviour is not widely studied; therefore, we might not find a

1 sufficient amount of reports to analyse; furthermore, we are focusing on five
2 polymorphisms and perhaps there are not enough reports of each one. Secondly,
3 the reports will be limited to English language journals, so the number of studies
4 might be too small. High heterogeneity may also result due to differences in age,
5 ethnicity, gender, education, wealth, etc. These limitations will decrease the
6 statistical power of the study and reliability of our results.

7 Conversely, this will be the first meta-analysis exploring the link between the five
8 SNPs of HTR2C and suicidal behaviour, specifically examining the allelic, additive,
9 dominant, and recessive models. In addition, this study protocol could demonstrate
10 the link between the 5-HTR2C gene and suicidal behaviour among the general
11 population.

12 Performing an analysis by gender is a strong point; in theory, due to its
13 chromosomal location (X chromosome), this gene must affect females and males
14 differently. The results could help to determine if HTR2C is a risk factor in
15 developing suicidal behaviour. Indirectly, our study could aid in understanding the
16 aetiology of suicidal behaviour.

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18 **Ethics and dissemination:** This study will describe the association between
19 HTR2C and suicidal behaviour. The results of this study will be reported in a peer-
20 reviewed publication and in scientific presentations in Mexico and throughout the
21 world.

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3 **1 Author contributions:**
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6 2 TZCA and GCTB conceived the study, participated in the design and helped to
7
8 3 draft the manuscript. GA, JRI and LNML helped on the selection of genes and
9
10 4 polymorphisms to study. TZCA, GCTB and A wrote the manuscript. All of the
11
12 5 authors read, critically revised and approved the final version of the manuscript.
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16 **6 Funding statement:**
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19 7 This research received no specific grant from any funding agency.
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22 **8 Competing interests statement:**
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25 9 The authors declare not to have any competing interests.
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29 **10 Figure legends**
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32 **11 Figure 1**
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35 12 Distribution of association studies on the 5-HTR2C gene and suicidal behaviour
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37 13 published in PubMed by year (figure adapted from the results of GoPubMed®).
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41 **14 Figure 2**
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43 15 Flow-chart for the search strategy and the inclusion/exclusion criteria used in the
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45 16 meta-analysis and systematic review.
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51 **18 Figure 3**
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54 19 World map of genetic association studies of the 5-HTR2C gene with suicidal
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56 20 behaviour (figure adapted from the results of GoPubMed®).
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**Association between HTR2C gene variants and suicidal behaviour: A
protocol for the systematic review and meta-analysis of genetic studies.**

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Abstract

Introduction: Suicide is an important public health problem and one of the most common causes of death throughout the world. Suicidal behaviour is complex, and its causes are multi-factorial. Case-control studies have reported an association between an alteration of the serotonin system and suicidal behaviour. Recently, it has been suggested that the 5-HT_{2C} serotonin receptor gene has been suggested to be involved in its pathogenesis of suicidal behaviour.

Methods and analysis: To evaluate the role of the 5-HT_{2C} gene in suicidal behaviour, we will perform a systematic review and a meta-analysis of world-wide reports that have investigated this association between the serotonin system and suicidal behaviour. This analysis will be reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) criteria. Studies deemed for inclusion in the systematic review will be scored for methodological quality using the Newcastle-Ottawa Assessment Scale (NOS). The inclusion criteria will be to present independent data, must be case-control studies, and to be published in journal peer-reviews. To generate more accurate analyses, we will grade the reports using the GRADES scale procedures.

Ethics and dissemination: This study will describe the association between the HTR_{2C} gene and suicidal behaviour. The results will be disseminated by reported in a peer-reviewed publication and in scientific presentations in Mexico and throughout the world.

Protocol registration: PROSPERO CRD42014009213

Comment [Ed1]: Perhaps "multi-faceted" would work better here.

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Strengths and limitations of the study

- The results could improve the understanding of the genetic component of suicidal behaviour.
- This study could show evidence of an association between the 5-HTR2C gene and suicidal behaviour.
- We will perform an analysis by gender that could determine if the 5-HTR2C gene affects females and males differently.
- A small amount-number of reports (and therefore a small sample size) is a possible limitation.
- High heterogeneity among the reports could be a limitation.

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Introduction

Suicide is the act of taking one's own life voluntarily and intentionally. Suicidal behaviour (SB) includes a wide spectrum of self-destructive actions that can be classified as: 1) suicidal ideation (thoughts of ending one's own life, including planning a specific method to succeed), 2) attempted suicide or parasuicide (when an individual deliberately hurts himself but the act does not result in death) and 3) completed suicide (when the action ends the life of the person who carries it out).¹⁻³

Suicide is a worldwide problem, and ~~it~~ ~~its prevalence is~~ ~~has been~~ increasing through over time.⁴⁻⁶ Social factors alone can not fully explain the suicidal behaviour.⁷⁻⁹ Furthermore, some studies have found that there is a potential genetic component ~~in it~~ ~~linked to suicidal behaviour~~.^{1,10}

The genetic studies that have tried to identify genes that predispose certain individuals to suicidal behaviour, have focused on genes that codify the proteins involved in the serotonergic system, ~~since because~~ this system interferes ~~in with~~ the control of mood, emotions, aggression and anxiety, ~~all of this again which~~ could influence suicidal behaviour.¹¹⁻¹³ Recently, ~~it has been proposed that~~ the 5-HTT2C gene ~~has been proposed to be~~ related to suicidal behaviour.^{10,14-17} due to its association with other mental diseases such as schizophrenia,¹⁸⁻²⁰ bipolar disorder,^{21,22} alcoholism,²³ and substance abuse.²⁴ Furthermore, the 5-HTT2C gene has been associated with anxiety, mood disorders and impulsiveness, and these are important suicide risk factors.^{16,25}

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1 ~~The~~ HTR2C (the 5-HTR2C gene) is a serotonin 2C receptor that lies on the X
 2 chromosome on the, long arm at, position Xq24-q28. ~~It~~ HTR2C has five introns
 3 and six exons, and so far, it is the only G-protein binding receptor known to
 4 undergo alternative splicing of the mRNA, therefore to have resulting in more
 5 protein isoforms.²⁶ ~~It~~ HTR2C has a vast number of single nucleotide
 6 polymorphisms (SNPs). More of than 1000 polymorphism have been identified
 7 along the gene (according to snpper.chip.org, October 2, 2013).²⁷ To date, the
 8 association between ~~the~~ HTR2C and suicidal behaviour ur remains controversial
 9 since because the reports include both positive and negative findings.²⁸⁻³² ~~F~~ The
 10 figure 1 shows the distribution of HTR2C and suicide studies over time.
 11 Consequently, it is important to perform a deeper study assessing the possible
 12 connection between the 5-HTR2C gene and suicidal behaviour ur.

14 Aims and objectives

15 This study aims to systematically review and ~~meta-analyze~~ perform a meta-analysis
 16 on the world-wide reports that have ~~researched for~~ investigated an association
 17 between the serotonin 2C receptor gene (5-HTR2C) and suicidal behaviour ur.
 18 Particularly, it aims to focus on rs547536, rs2192372, rs4272555, rs24287207 and
 19 rs6318 polymorphisms in order to detect which ones act as a possible risk factors s.

21 Methods/analysis

22 Systematic Review and Meta-analysis

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1 This meta-analysis will be reported following the Preferred Reporting Items for
2 Systematic Reviews and Meta-Analyses (PRISMA) criteria.³³

3 Identification and selection of publications

4 For both, the systematic review and the meta-analysis, the literature will be
5 searched from three databases: PubMed, Web of Science and EBSCO
6 EBSCO—using the following keywords: “HTR2C and suicidal behaviour” and
7 “HTR2C and suicide”. These words will be combined to retrieve the summaries.

8 The selected publications selected will meet the following criteria: (1) to be
9 published in peer-reviewed journals, (2) to contain independent data, (3) to be
10 case-control association studies in which the frequencies of the three genotypes
11 are clearly stated or could be calculated, (4) the use of healthy individuals or
12 psychiatric patients without suicidal behaviour as controls; (5) to include one or
13 more of the three forms of suicidal behaviour (suicidal ideation, attempted suicide
14 or completed suicide); and (6) to be written in English. The procedure planned
15 procedure is exemplified-illustrated on-in figure-Figure 2.

16 To determine inclusion, two researchers (González-Castro and Juárez-Rojop) will
17 work independently to screening each of the titles, abstracts and full texts. When
18 the researchers disagree a third researcher (Tovilla-Zárate) will be consulted.

20 Data extraction

21 For the systematic review, the following data will be searched and

1 | ~~analyzed~~analysed: authors, year of publication, region, sample size, number of
2 | cases and controls, gender, age, psychiatric diagnosis, ethnical origin and finally,
3 | genotype and allele frequencies. The SNPs in the study will be rs547536,
4 | rs2192372, rs4272555, rs24287207 and rs6318.

5 | The meta-analysis will be constructed following four categories: (a) exposed sick,
6 | (b) exposed not-sick, (c) not-exposed sick and (d) not- exposed not-sick. The term
7 | “sick” ~~term~~ refers to subjects exhibiting suicidal behaviour and the term “exposed”
8 | refers term to the allele of risk.

9 | The data extraction will be performed by González-Castro, Juárez-Rojop, Genis-
10 | Mendoza and López-Nárvaez.

11 | **Data analysis**

12 | The systematic review ~~would~~will be presented ~~on~~in tables comparing quality
13 | measurements and the data previously mentioned. Studies deemed for inclusion
14 | will be scored for methodological quality using the Newcastle-Ottawa Assessment
15 | Scale (NOS).³⁴ A score of six will be considered ~~as~~ the cut-off point to distinguish
16 | higher from lower quality studies. To give more support to our analysis, we will
17 | consider the GRADES scale procedures (<http://www.gradeworkinggroup.org>).

18 | For the meta-analysis, we will use the EPIDAT 3.1 program (Software for
19 | Epidemiologic Analysis of Tabulated Data, Version 3.1, Pan American Health
20 | Organization; <http://dxsp.sergas.es>); this software is freely available for the
21 | epidemiologic analysis of tabulated data. Data will be ~~analyzed~~analysed using the
22 | random-effects model following the reports in the literature.³⁵⁻³⁷ The sample

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1 | heterogeneity will be measured ~~with-using~~ the Dersimonian and Laird's Q test; we
2 | will also use graphs to help ~~visualize—visualise~~ those studies favouring
3 | heterogeneity. The results of the meta-analysis will be expressed as odds ratios
4 | (ORs). Pooled ORs will be calculated for each model ~~used~~; viz.: allelic (Example:
5 | A vs. C), additive (AA vs. CC), dominant (AA + AC vs. CC), and recessive (AA vs.
6 | AC + CC). ~~Since-Because~~ the HTR2C ~~gene~~ is located on the X chromosome, an
7 | analysis by gender will be performed. To address the publication bias, the Egger's
8 | test and funnel plots will be calculated with the EPIDAT 3.1 software. This plotting
9 | will standardize the effect of each study on the vertical axis and its correspondent
10 | precision on the horizontal axis. Sensibility analysis will be performed. The
11 | influence of each study in the global estimation will be ~~analyzed—analysed~~ and
12 | graphed. The direction of the effect will be ~~analyzedanalysed~~. To evaluate
13 | genotype distribution and calculate the Hardy-Weinberg equilibrium, we will use a
14 | chi-squared (χ^2) analysis. Regarding the presence of Hardy-Weinberg equilibrium,
15 | we will use the Pearson's chi-squared test to assess goodness of fit. Finally, the
16 | Haploview 4.2 ³⁸ will be used to calculate the linkage disequilibrium (LD) of the
17 | markers.

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18 | The ~~analyzed—analysed~~ of dates will be performed by Gonzalez-Castro and Tovilla-
19 | Zarate- ~~Any d~~Differences in the interpretation ~~could—will~~ be resolved by López-
20 | Narvaez.

22 | Discussion

1 Suicide is a public health problem that affects entire communities and not ~~only-just~~
 2 the suicidal person. The medical costs and lost wages associated with suicide also
 3 take their toll on the community. Therefore, it is crucial to better understand the
 4 ~~etiology~~~~aetiology~~ of suicidal behaviour~~ur~~, as well as ~~knowing-define~~ how ~~big- large~~
 5 the problem is and whom it affects.

6 ~~The~~ literature suggests that there could be a genetic factor linked to suicide.
 7 Some studies have proposed a hypothetical explanation using the Stress-Diathesis
 8 Model and have observed that people who ~~have-had~~~~exhibit~~ suicidal behaviour~~ur~~ are
 9 more likely to have a family history of suicide.¹⁶ Therefore, ~~we can~~~~this finding~~
 10 ~~suggests~~ that a genetic component could contribute to suicidal tendencies.¹⁶ The
 11 serotonin neurotransmission system has received the most consideration as ~~the~~
 12 candidate genes ~~linked to~~ suicidal behaviour ~~because~~~~since~~ the serotonin system
 13 regulates mood, impulsiveness and anxiety, which are important suicide risk
 14 factors. Furthermore, the serotonergic function is clearly altered in suicide
 15 victims.¹⁰ Among the most studied genes, are the ones that codify ~~to the~~
 16 tryptophan hydroxylase 1 and 2 (TPH-1 and TPH-2), ~~the~~ serotonin transporter (5-
 17 HTT or SLC6A4) and the serotonin receptors (5-HTR1A, 5-HTR1B, 5-HTR2A, 5-
 18 HTR2C, etc.).³⁹ ~~Figure 3 summarizes the worldwide studies linking suicidal~~
 19 ~~behaviour with HTR2C, Being the gene last most recently one the most~~
 20 ~~recently highlighted in the literature in association with suicidal behaviour suggested~~
 21 ~~around the world to be associated with suicidal behavior Figure 3.~~ The ~~gene~~ 5-
 22 HTR2C ~~gene needs-requires~~ a deeper analysis; therefore, by ~~p~~Performing a
 23 systematic review and a meta-analysis, this protocol could improve the

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1 understanding of the genetic component in suicidal behaviour. ~~Regarding the~~
 2 data analysis, we will adopt the random effects model, because it accounts for
 3 additional sources of inter-study variation when heterogeneity exists and, ~~thus,~~
 4 a more reliable outcome.

5 This meta-analysis could present some limitations. First, the association between
 6 HTR2C and suicidal behaviour is not widely studied; therefore, we might not find a
 7 sufficient amount of reports to ~~analyze~~analyse; furthermore, we are focusing on
 8 five polymorphisms and perhaps there are not enough reports of each one.
 9 Secondly, the reports will be limited to English language journals, so the number of
 10 studies might be too small. High heterogeneity ~~may also result,~~ due to differences
 11 in age, ethnicity, gender, education, wealth, etc. These limitations will decrease the
 12 statistical power of the study ~~and~~ reliability of our results.

13 ~~Conversely, it—this would—will be the first meta-analysis associating~~
 14 ~~between exploring the link between the~~ five SNPs of HTR2C and suicidal
 15 behaviour, ~~specifically examining the with~~ allelic, additive, dominant, and recessive
 16 models. ~~In addition, this study protocol could demonstrate the link between the 5-~~
 17 HTR2C ~~gene in the~~and suicidal behaviour ~~of among~~ the general population.

18 Performing an analysis by gender is a strong point; in theory, due to its
 19 chromosomal location (X chromosome), this gene must affect females and males
 20 differently. The results could help to determine if ~~the~~HTR2C ~~gene~~ is a risk factor in
 21 developing suicidal behaviour. Indirectly, our study could ~~help to aid in~~
 22 understanding the ~~etiologyaetiology~~ of suicidal behaviour.

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Ethics and dissemination: This study will describe the association between HTR2C gene and suicidal behavior. The results of this study will be disseminated reported in by a peer-reviewed publication and in scientific presentations in Mexico and throughout the world.

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2 | **Author's contributions:**

3 | TZCA and GCTB conceived the study, participated in the design and helped to
4 | draft the manuscript. GA, JRI and LNML helped on the selection of genes and
5 | polymorphisms to study. TZCA, GCTB and A wrote the manuscript. All [of the](#)
6 | authors read, critically revised and approved the final version of the manuscript.

7

8 | **Funding statement:**

9 | This research received no specific grant from any funding [agency](#).

10 | **Competing interests statement:**

11 | The authors declare not to have any competing interests.

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10 **Figure legends**
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13 Figure 1

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16 Distribution of association studies on the 5-HTR2C gene and suicidal behaviour
17 published in PubMed by year ~~(f~~Figure adapted from the results of GoPubMed®).
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20 ~~Figure 2-~~

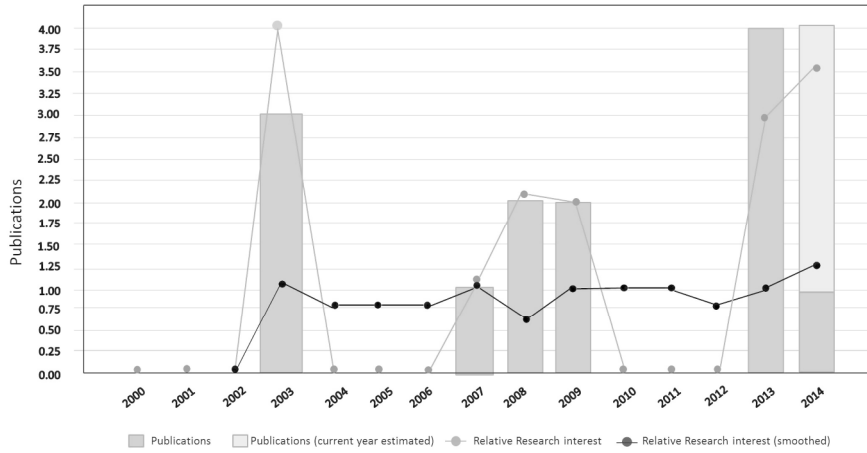
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22 Flow-chart for the search strategy and the inclusion/exclusion criteria ~~to be~~ used in
23 the meta-analysis and systematic review.
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29 Figure 3

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31 World map of ~~Genetic-genetic~~ association studies of the 5-HTR2C gene with
32 suicidal behaviour ~~(f~~Figure adapted from the results of GoPubMed®).
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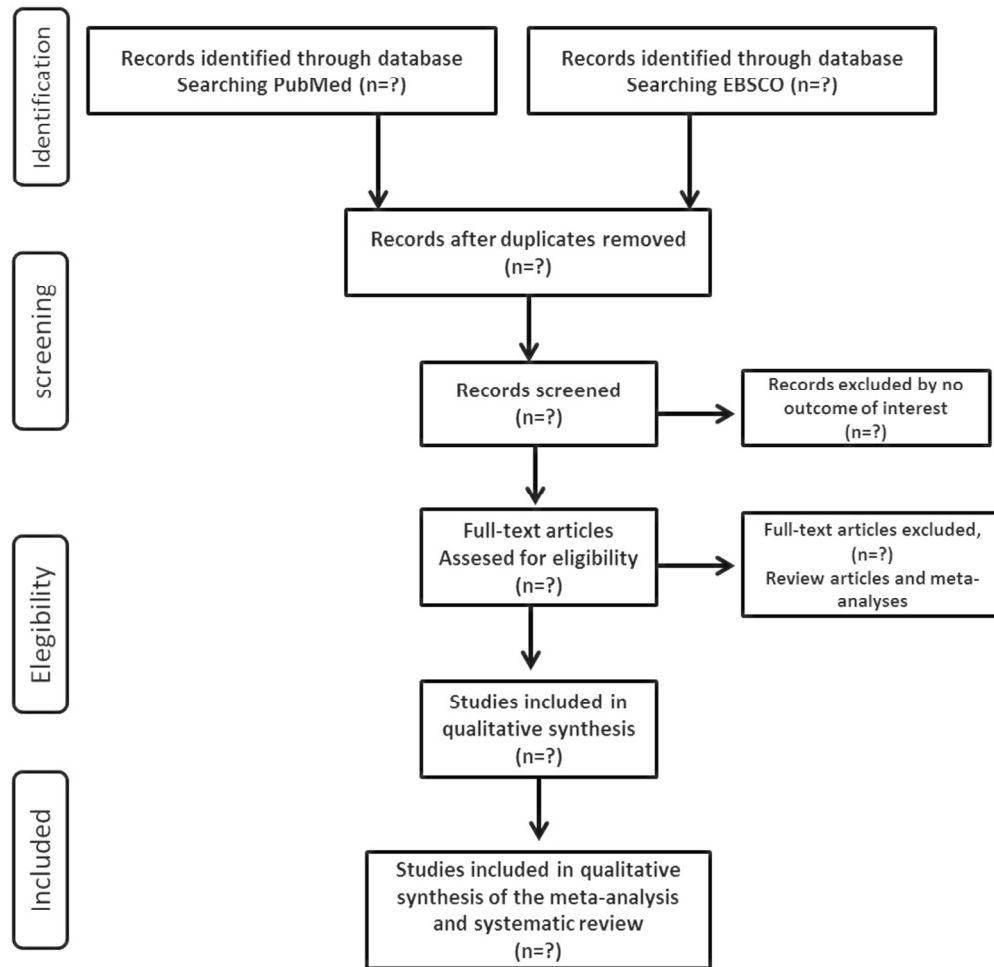
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Distribution of association studies on the 5-HTR2C gene and suicidal behaviour published in PubMed by year (figure adapted from the results of GoPubMed®).
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Flow-chart for the search strategy and the inclusion/exclusion criteria used in the meta-analysis and systematic review.
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World map of genetic association studies of the 5-HTT2C gene with suicidal behaviour (figure adapted from the results of GoPubMed®).

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