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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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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-HTRC 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-HTR2C 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

Strength and limitations of this study

- This study could improve the understanding of the genetic component of suicidal behavior.
- This study protocol could show evidence of the role that the 5-HTR2C gene plays in the suicidal behavior.
- A small sample or the presence of high heterogeneity could be a limitation.

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 tied to find and explain genetic causes of the disorder, because social factors alone can not explain completely the suicidal behavior.[7]

The receptor 5HTR2C 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-HT2C has been implicated in numerous brain functions, regulation of locomotion, sex, instinct and appetite to modulation of

mood and anxiety among others.[9] 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.[10] The HTR2C (5-HT2C gene) has a vast number of single nucleotide polymorphisms (SNPs). More of 1000 polymorphism have been identified along the gene (according to snpper.chip.org, October 2, 2013).[11] This gene has been associated with many diseases as schizophrenia,[12 13] weigh gain in schizophrenia,[14] bipolar disorder,[15 16] alcoholism,[17] and substance abuse,[18] However, the association between HTR2C and suicidal behavioral remains controversial, given that to date case-control studies include positive and negative findings.[19-23]

Aims and objectives

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

Methods/analysis

Meta-analysis

This meta-analysis will follow the Preferred Reporting Items for Systematic

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

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

Discussion

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. It is well known that suicide not only affects the person who commits it, is also affects the health of the community; family and friends of people who commit suicide may experience shock, anger,

guilt, and depression. The medical costs and lost wages associated with suicide also take their toll on the community. It has been proposed the importance of the analysis of the suicide; but before we can prevent suicide, we need to know how big the problem is, where it is, and whom it affects.

Besides the studies which reveal social factor that increase the possibility to commit a suicide, there are others that support that it could exist a genetic link to suicide; in these studies we observe that people who completed suicide or who have had suicidal thoughts or behavior are more likely to have a family history of suicide. Therefore we can suggest that more studies are necessary to understand a possible genetic component that could contribute to suicidal tendencies [29]. The 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 victims.[30] The most studied genes are the tryptophan hydroxylase 1 and 2 (TPH-1 and TPH-2), serotonin transporter (5-HTT or SLC6A4) and the serotonin receptors (5-HTR1A, 5-HTR1B, 5-HTR2A, 5-HTR2C, etc).[31] Being the last one the most recently studied in this area around the world Figure 2. The gene 5-HTR2C is an excellent candidate, although its study needs a detailed analysis because its chromosomal location.[29] Also, a systematic review and meta-analysis could improve the understanding of a genetic component in suicidal behavior. This study protocol could show evidence of the role that the 5-HTR2C plays in the suicidal behavior in all population with an up-to-date meta-analysis and systematic review with the existing publications.

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.

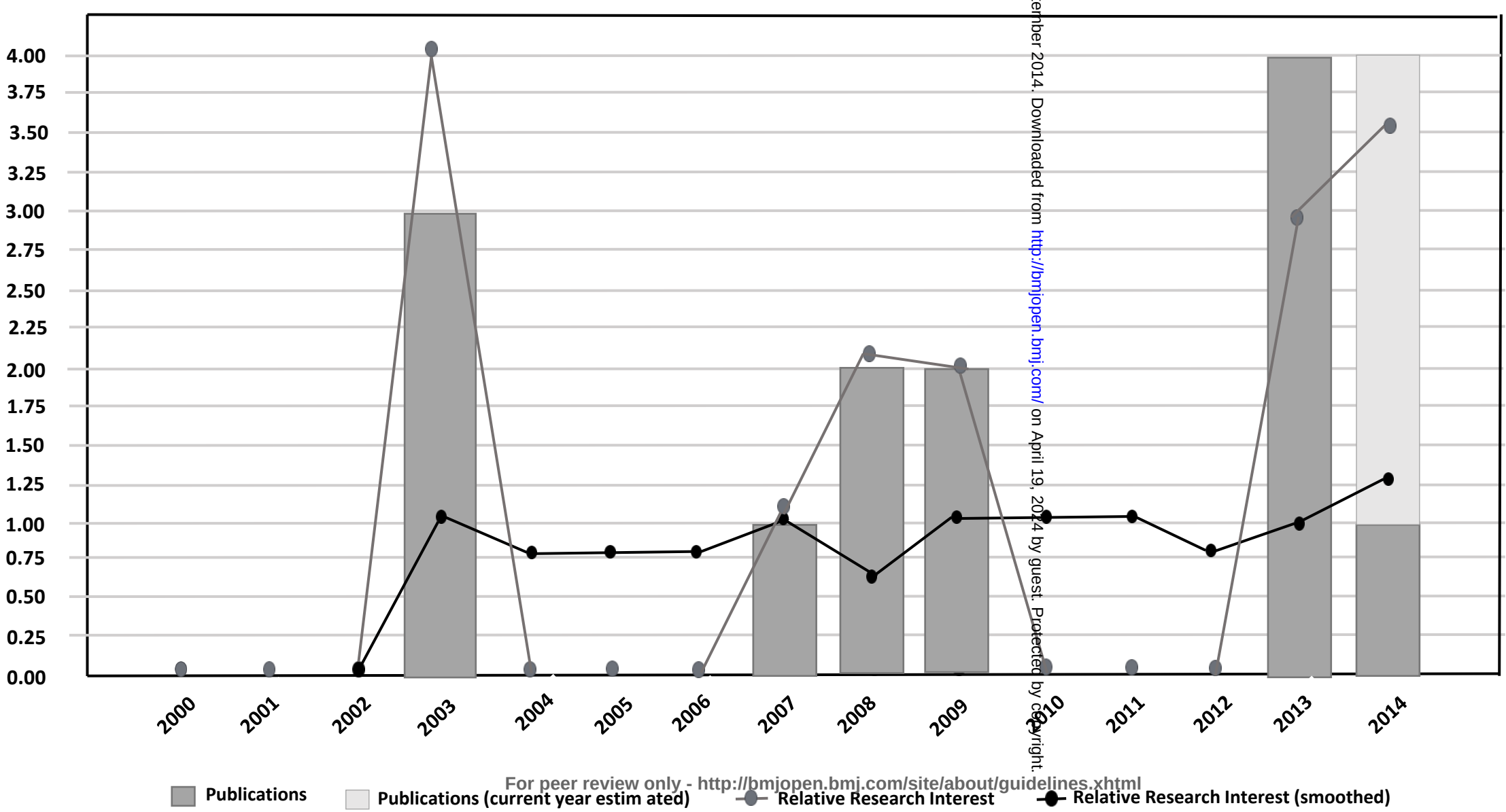
Figure legends

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)



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

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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 behavior is complex and has multi-factorial causes; case-control studies have reported an association between an alteration of the serotonin system and suicidal behavior; recently, the 5-HTRC2 serotonin receptor gene has been suggested to be involved in its pathogenesis.

***Methods and analysis*:** To evaluate the role of the 5-HTR2C gene in suicidal behavior, we will perform a systematic review and a meta-analysis of world wide reports that have investigated this association. 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 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 HTR2C gene and suicidal behavior. The results will be disseminated by a peer-reviewed publication and scientific presentations in Mexico and throughout the world.

Protocol registration: PROSPERO CRD42014009213

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.

Introduction

Suicide is the act of taking one's own life voluntarily and intentionally. Suicidal behavior (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 is increasing through time.[4-6] Social factors alone can not fully explain the suicidal behavior,[7-9] furthermore, some studies have found that there is a potential genetic component in it.[1 10]

The genetic studies that have tried to identify genes that predispose to suicidal behavior, have focused on genes that codify proteins involved in the serotoninergic system, since this system interferes in controlling mood, emotions, aggression and anxiety, this again could influence suicidal behavior.[11-13] Recently, the 5HTR2C gene has been proposed to be related to suicidal behavior,[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 5HTR2C gene has been associated with anxiety, mood disorders and impulsiveness, and these are important suicide risk factors.[16 25]

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

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

Aims and objectives

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

Methods/analysis

Systematic Review and Meta-analysis

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

Identification and selection of publications

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

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

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

Data extraction

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

rs24287207 and rs6318.

The meta-analysis will be constructed following four 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.

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

Data analysis

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

For the meta-analysis we will use the EPIDAT 3.1 program (Software for Epidemiologic Analysis of Tabulated Data. Version 3.1. Pan American Health Organization; http://dxsp.sergas.es); this software is freely available for epidemiologic analysis of tabulated data. Data will be analyzed using the random-effects model following the reports in literature [35-37]. The sample heterogeneity will be measured with the Dersimonian and Laird's Q test; we will also use graphs to help visualize those studies favoring heterogeneity. The results of the meta-analysis will be expressed as odds ratios (ORs). Pooled ORs will be calculated for

each model used, viz.: allelic (Example: A vs. C), additive (AA vs. CC), dominant (AA + AC vs. CC), and recessive (AA vs. AC + CC). Since the HTR2C gene is located on the X chromosome, an analysis by gender will be performed. To address the publication bias, the Egger's test and funnel plots will be calculated with the EPIDAT 3.1 software. This plotting will standardize the effect of each study on the vertical axis and its correspondent precision on the horizontal axis. Sensibility analysis will be performed. The influence of each study in the global estimation will be analyzed and graphed. The direction of the effect will be analyzed. To evaluate genotype distribution and calculate the Hardy-Weinberg equilibrium, we will use a chi-squared (χ^2) analysis. Regarding the presence of Hardy-Weinberg equilibrium, we will use the Pearson's chi-squared test to assess goodness of fit. Finally, the Haploview 4.2 [38] will be used to calculate the linkage disequilibrium (LD) of the markers.

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

Discussion

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

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

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

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

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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 by peer-reviewed publication and scientific presentations in Mexico and throughout the world.

Author's contribution:

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

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The authors declare not to have any competing interests.

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Figure legends

Figure 1

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

Figure 2.

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

Figure 3

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

Association between HTR2C gene variants and suicidal behavior: protocol for 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 behavior is complex and has multi-factorial causes; case-control studies have reported an association between an alteration of the serotonin system and suicidal behavior; recently, the 5-HTRC2 serotonin receptor gene has been suggested to be involved in its pathogenesis.

***Methods and analysis*:** To evaluate the role of the 5-HTR2C gene in suicidal behavior, we will perform a systematic review and a meta-analysis of world wide reports that have investigated this association. 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 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 HTR2C gene and suicidal behavior. The results will be disseminated by a peer-reviewed publication and scientific presentations in Mexico and throughout the world.

Protocol registration: PROSPERO CRD42014009213

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.

Introduction

Suicide is the act of taking one's own life voluntarily and intentionally. Suicidal behavior (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 is increasing through time.[4-6] Social factors alone can not fully explain the suicidal behavior,[7-9] furthermore, some studies have found that there is a potential genetic component in it.[1 10]

The genetic studies that have tried to identify genes that predispose to suicidal behavior, have focused on genes that codify proteins involved in the serotoninergic system, since this system interferes in controlling mood, emotions, aggression and anxiety, this again could influence suicidal behavior.[11-13] Recently, the 5HTR2C gene has been proposed to be related to suicidal behavior,[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 5HTR2C gene has been associated with anxiety, mood disorders and impulsiveness, and these are important suicide risk factors.[16 25]

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

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

Aims and objectives

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

Methods/analysis

Systematic Review and Meta-analysis

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

Identification and selection of publications

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

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

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

Data extraction

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

rs24287207 and rs6318.

The meta-analysis will be constructed following four 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.

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

Data analysis

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

For the meta-analysis we will use the EPIDAT 3.1 program (Software for Epidemiologic Analysis of Tabulated Data. Version 3.1. Pan American Health Organization; http://dxsp.sergas.es); this software is freely available for epidemiologic analysis of tabulated data. Data will be analyzed using the random-effects model following the reports in literature [35-37]. The sample heterogeneity will be measured with the Dersimonian and Laird's Q test; we will also use graphs to help visualize those studies favoring heterogeneity. The results of the meta-analysis will be expressed as odds ratios (ORs). Pooled ORs will be calculated for

each model used, viz.: allelic (Example: A vs. C), additive (AA vs. CC), dominant (AA + AC vs. CC), and recessive (AA vs. AC + CC). Since the HTR2C gene is located on the X chromosome, an analysis by gender will be performed. To address the publication bias, the Egger's test and funnel plots will be calculated with the EPIDAT 3.1 software. This plotting will standardize the effect of each study on the vertical axis and its correspondent precision on the horizontal axis. Sensibility analysis will be performed. The influence of each study in the global estimation will be analyzed and graphed. The direction of the effect will be analyzed. To evaluate genotype distribution and calculate the Hardy-Weinberg equilibrium, we will use a chi-squared (χ^2) analysis. Regarding the presence of Hardy-Weinberg equilibrium, we will use the Pearson's chi-squared test to assess goodness of fit. Finally, the Haploview 4.2 [38] will be used to calculate the linkage disequilibrium (LD) of the markers.

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

Discussion

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

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

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

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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 on the selection of genes and polymorphisms to study. TZCA, GCTB and A wrote the manuscript. All authors read, critically revised and approved the final version of the manuscript.

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Figure legends

Figure 1

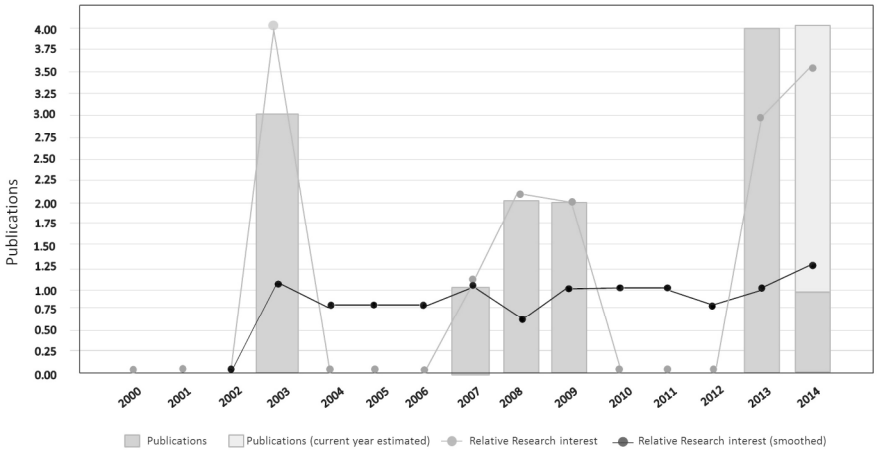
Representation of 5-HTR2C gene studies over time. (All data from: www.gopubmed.com)

Figure 2.

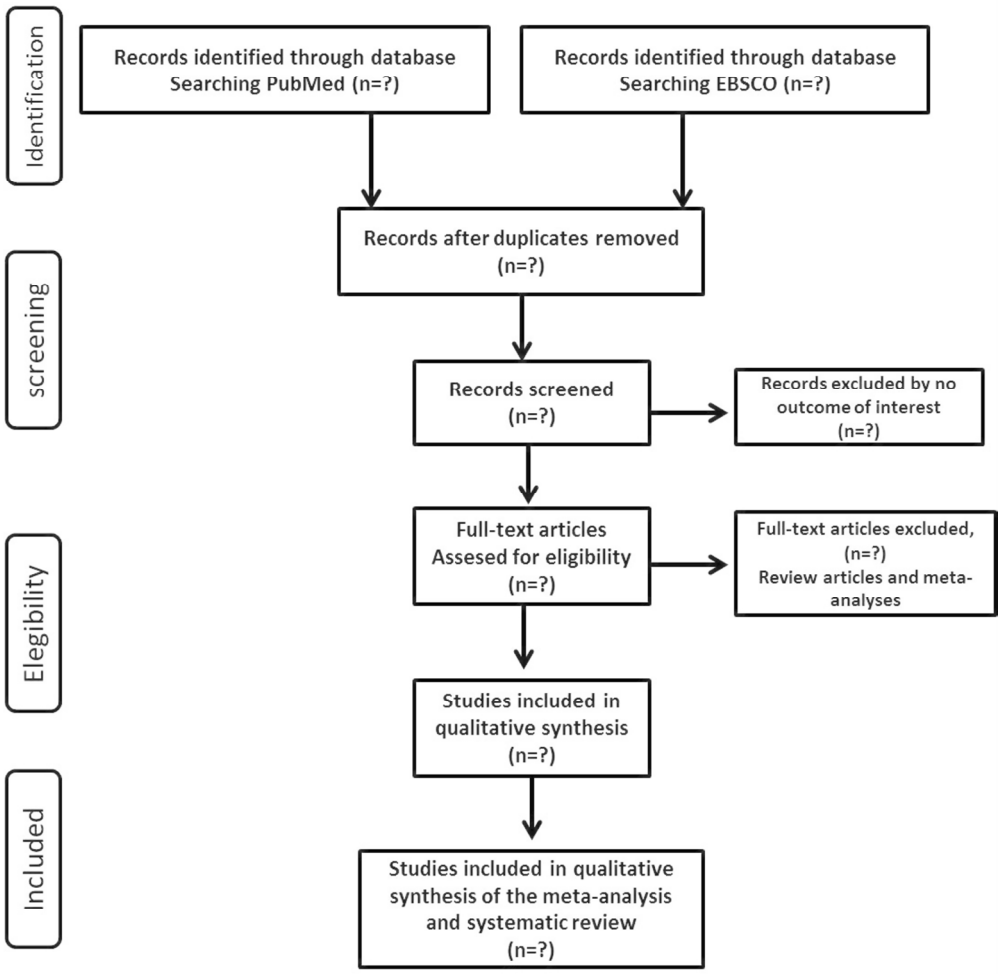
Flow-chart for the search strategy and the inclusion/exclusion criteria to be used in the meta-analysis and systematic review.

Figure 3

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



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)



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

World map of genetic association studies of the 5-HTR2C gene with suicidal 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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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-HTRC2 serotonin receptor gene is involved in the pathogenesis of suicidal behaviour.

***Methods and analysis*:** To evaluate the role of the 5-HTR2C 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 HTR2C 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

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.

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 its prevalence has been increasing over time.[4-6] Social factors alone cannot fully explain suicidal behaviour.[7-9] Furthermore, some studies have found that there is a potential genetic component 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 serotoninergic system because this system interferes with the control of mood, emotions, aggression and anxiety, all of which could influence suicidal behaviour.[11-13] Recently, it has been proposed that the 5-HTR2C gene is 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-HTR2C gene has been associated with anxiety, mood disorders and impulsiveness, and these are important suicide risk factors.[16 25]

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

Aims and objectives

This study aims to systematically review and perform a meta-analysis on the worldwide reports that have investigated an association between the serotonin 2C receptor gene (5-HTR2C) and suicidal behaviour. Particularly, it aims to focus on rs547536, rs2192372, rs4272555, rs24287207 and rs6318 polymorphisms to detect which ones act as possible risk factors.

Methods/analysis

Systematic Review and Meta-analysis

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

Identification and selection of publications

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

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

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

Data extraction

For the systematic review, the following data will be searched and analysed: authors, year of publication, region, sample size, number of cases and controls,

gender, age, psychiatric diagnosis, ethnical origin and finally, genotype and allele frequencies. The SNPs in the study will be rs547536, rs2192372, rs4272555, rs24287207 and rs6318.

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

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

Data analysis

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

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

to help visualise those studies favouring heterogeneity. The results of the meta-analysis will be expressed as odds ratios (ORs). Pooled ORs will be calculated for each model used: viz.: allelic (Example: A vs. C), additive (AA vs. CC), dominant (AA + AC vs. CC), and recessive (AA vs. AC + CC). Because the HTR2C is located on the X chromosome, an analysis by gender will be performed. To address the publication bias, the Egger's test and funnel plots will be calculated with the EPIDAT 3.1 software. This plotting will standardize the effect of each study on the vertical axis and its correspondent precision on the horizontal axis. Sensibility analysis will be performed. The influence of each study in the global estimation will be analysed and graphed. The direction of the effect will be analysed. To evaluate genotype distribution and calculate the Hardy-Weinberg equilibrium, we will use a chi-squared (χ^2) analysis. Regarding the presence of Hardy-Weinberg equilibrium, we will use the Pearson's chi-squared test to assess goodness of fit. Finally, the Haploview 4.2 [38] will be used to calculate the linkage disequilibrium (LD) of the markers.

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

Discussion

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

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

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

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

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

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

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

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

Author contributions:

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

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Competing interests statement:

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Figure legends

Figure 1

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-HTR2C gene with suicidal 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 ~~has~~ multi-factorial. ~~causes; C~~case-control studies have reported an association between an alteration of the serotonin system and suicidal behaviour. ~~;~~ R~~r~~ecently, it has been suggested that the 5-HTRC2 serotonin receptor gene ~~has been suggested to be~~is involved in ~~its~~ the pathogenesis of suicidal behaviour.

Methods and analysis: To evaluate the role of the 5-HTR2C gene in suicidal behaviour, we will perform a systematic review and a meta-analysis of world~~-~~wide reports that have investigated ~~this~~ 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, ~~must~~ 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 HTR2C 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

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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] F~~f~~urthermore, 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 serotoninergic system~~, since~~ because this system interferes ~~in~~ with the control of~~ling~~ mood, emotions, aggression and anxiety, all of ~~this again~~which could influence suicidal behaviour.[11-13] Recently, it has been proposed that the 5-HTR2C gene ~~has been proposed to be~~is 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-HTR2C gene has been associated with anxiety, mood disorders and impulsiveness, and these are important suicide risk factors.[16 25]

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

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Aims and objectives

This study aims to systematically review and meta-analyzeperform a meta-analysis on the world-wide reports that have researched forinvestigated an association between the serotonin 2C receptor gene (5-HTR2C) and suicidal behaviour. Particularly, it aims to focus on rs547536, rs2192372, rs4272555, rs24287207 and rs6318 polymorphisms in order to detect which ones act as a possible risk factors.

Methods/analysis

Systematic Review and Meta-analysis

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

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Identification and selection of publications

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

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

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

Data extraction

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

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

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

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

Data analysis

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

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

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

The ~~analyzed~~ analysed of dates will be performed be Gonzalez-Castro and Tovilla-Zarate- Any d~~D~~ifferences in the interpretation ~~could~~ will be resolved by López-Narvaez.

Discussion

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

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

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

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

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

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

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Ethics and dissemination: This study will describe the association between HTR2C ~~gene~~ and suicidal behaviour. 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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Author~~'s~~ contributions:

TZCA and GCTB conceived the study, participated in the design and helped to draft the manuscript. GA, JRI and LNML helped on the selection of genes and polymorphisms to study. TZCA, GCTB and A wrote the manuscript. All of the 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

Distribution of association studies on the 5-HTR2C gene and suicidal behaviour published in PubMed by year . (fFigure adapted from the results of GoPubMed®).

Figure 2

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

Figure 3

World map of Genetic genetic association studies of the 5-HTR2C gene with suicidal behaviour (fFigure adapted from the results of GoPubMed®).

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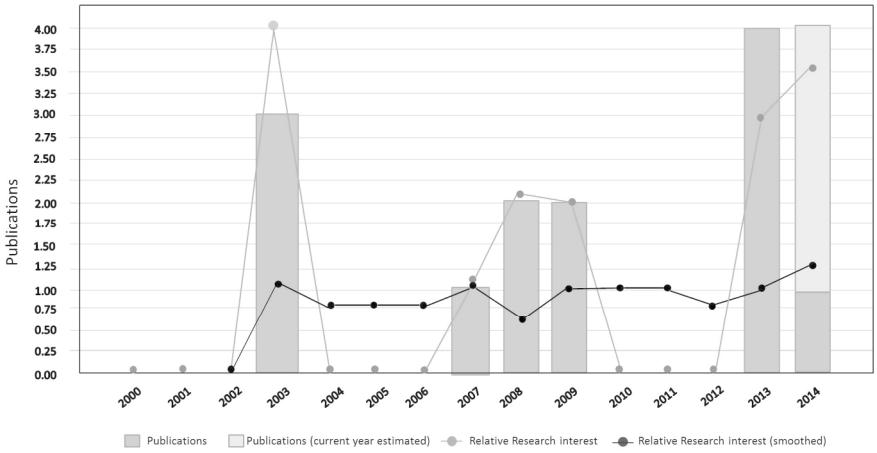

Distribution of association studies on the 5-HTR2C gene and suicidal behaviour published in PubMed by year (figure adapted from the results of GoPubMed®).
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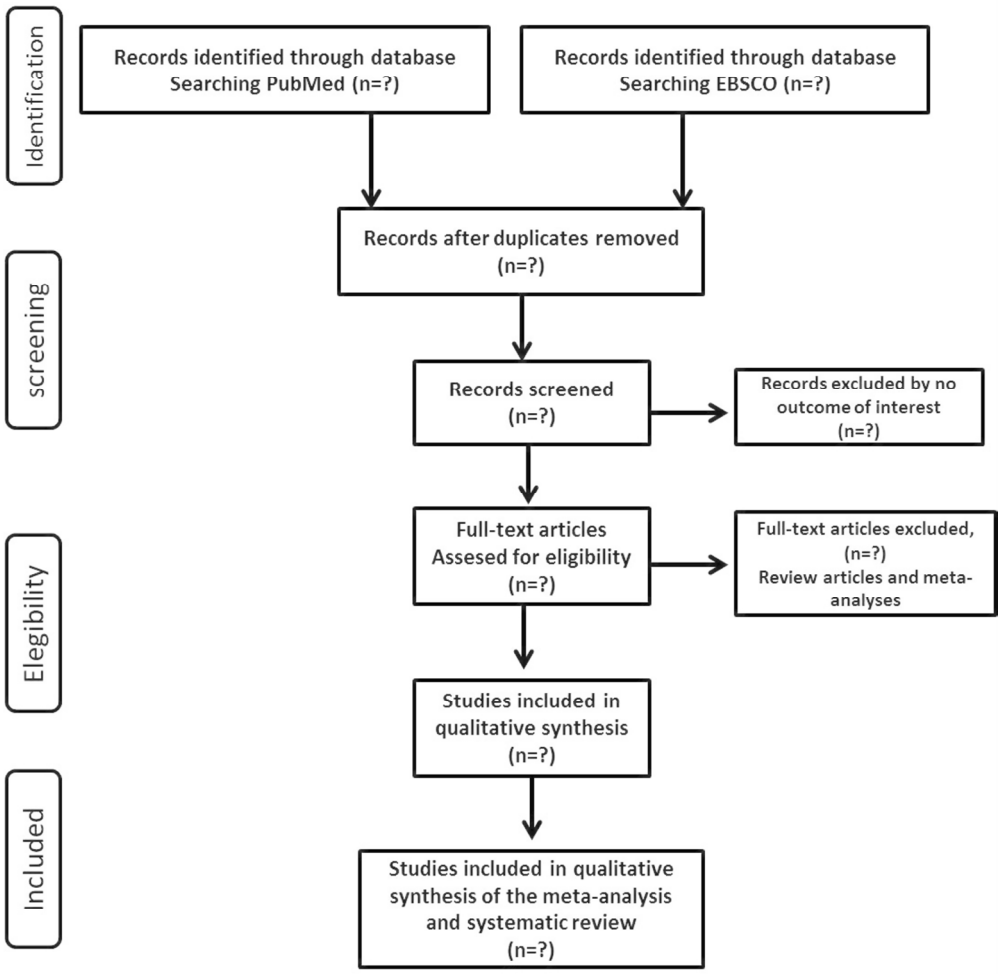

Flow-chart for the search strategy and the inclusion/exclusion criteria used in the meta-analysis and systematic review.
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World map of genetic association studies of the 5-HTR2C gene with suicidal behaviour (figure adapted from the results of GoPubMed®).