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# Genome-wide association study of suicide behavior in psychiatric disorders: A protocol in Mexican population



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# Genome-wide association study of suicide behavior in psychiatric disorders: A protocol in Mexican population

Thelma Beatriz González Castro[1a], Carlos Alfonso Tovilla-Zarate[2a], José Jaime Martínez-Magaña[3], Isela Esther Juarez-Rojop[4], Emmanuel Sarmiento[5], Alma Delia Genis-Mendoza*[3,5], Humberto Nicolini*[3]

[1] División Académica Multidisciplinaria de Jalpa de Méndez; Universidad Juárez Autónoma de Tabasco. Jalpa de Méndez, Tabasco, México.

[2] División Académica Multidisciplinaria de Comalcalco; Universidad Juárez Autónoma de Tabasco. Comalcalco, Tabasco, México.

[3] Instituto Nacional de Medicina Genómica (INMEGEN); Secretaria de Salud. Ciudad de México.

[4] División Académica Multidisciplinaria de Ciencias de la Salud; Universidad Juárez Autónoma de Tabasco. Villahermosa, Tabasco, México.

[5] Hospital Psiquiátrico Infantil "Dr. Juan N. Navarro". Ciudad de México

[a] these authors contributed equally to this work

TBGC: thelma.glez.castro@gmail.com; CATZ: alfonso_tovillaz@yahoo.com.mx; JJMM: jimy.10.06@gmail.com; IEJR: iselajuarezrojop@hotmail.com; ES: emmanuelsarmientoh@hotmail.com

*Correspondence authors: Ph.D. Alma Genis-Mendoza, National Institute of Genomic Medicine (INMEGEN), Psychiatric Care Services (SAP). Periférico Sur 4809, Arenal Tepepan, 14610, Mexico City, Mexico. Phone + 55 5350 1900. Email address: adgenis@inmegen.gob.mx or Humberto Nicolini: hnicolini@inmegen.gob.mx

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## Abstract

*Introduction*: Suicidality is a complex behavior and major health problem, in which the specific features that could predispose to suicidal behavior have been extensively investigated most frequently in Europeans and Asians. Due to this, our aim is to present a protocol that will explore the suicide attempt in psychiatric disorders by performing a genome-wide association study in a Mexican population.

*Method and analysis*: 700 Mexican cases diagnosed as schizophrenics with or without a suicide attempt and bipolar with or without a suicide attempt; also suicide attempters without psychiatric comorbidity will be analyzed. The genotyping will be performed using the Infinium PsychArray BeadChip, following the manufacturer's protocol.

*Ethics and dissemination*: This type of genetic tools will allow us to identify the associated SNPs in mental illnesses such as schizophrenia and bipolar disorder and their possible participation as predictors of suicidal behavior in a Mexican population, so the outcomes of the study derived of the current protocol are essential data to get a better comprehension of SB. This study has been approved by the ethics and investigation commitments of the National Institute of Genomic Medicine

*Trial registration*: CEI 215/13


Keywords: suicide & self-harm; mental health; schizophrenia & psychotic disorders; genetics

## Article summary

### Strengths and limitations of this study

Our results would be the first report in a Mexican population to perform a GWAS in association with suicide.

These outcomes would allow identifying candidate variants for schizophrenia and bipolar disorder in Mexico as predictors for SB.

Mexican population has a heterogeneous genetic background, which could interfere in the interpretation of the results.

The findings would provide helpful information, so that in the future the Latin population could be considered in the design of microarrays.

## Introduction

Suicide is one of the leading causes of death worldwide. Individuals diagnosed with a psychiatric disorder have higher rates of suicide compared to the general population, which emphasizes a narrow relationship between suicide and psychiatric disorders. In this line of evidence, a considerable majority of suicide victims have had an undiagnosed psychiatric disorder at the time of death [1-3]. Furthermore, several studies consider psychiatric disorders as one of the main risk factors of suicide. Unfortunately, up to today, the mechanisms that explain this relationship have not been fully disentangled [4 5].

Suicidal behavior (SB), has been defined as a complex issue that results from the combination of genetic variants, along with personal experiences and environmental contribution; altogether, these factors establish the disease symptomatology manifestation [1, 3, 12]. The results of studies based on twins, adoptions and families, support the heritability of SB, pinpointing a genetic background [6-9]. Subsequently, many candidate-gene association studies have been performed, with the different phenotypes of SB: suicide attempts, suicide ideation and accomplished suicide [1 3 10-12]. Unfortunately, SB is a polygenic trait and candidate-gene association studies underestimate the genetic background. In an attempt to search for this polygenic variation of SB, other strategies have been developed, such as the genome-wide association studies (GWAS) [12-14].

Over the past decade, a small number of GWAS, exploring common genetic variation mostly in suicide attempters have found significant associations between genetic components and SB, establishing possible molecular pathways involved in

the susceptibility of the disease [15 16]. However, even if a genetic diathesis model for predisposition to SB has been proposed and GWAS have suggest candidate loci or pathways, the only information published available comes from studies analyzing Caucasians or Asians populations, leaving behind Latin American populations including Mexicans [17-21]. Therefore, more studies are necessary in order to have a better comprehension of the genetic background of SB.

Objective

Our aim is to present a protocol for the first genome-wide association study of suicide attempters in a Mexican population, in order to explore and define the involvement of a genetic diathesis that predispose to SB in Mexicans.

**Methods and analysis**

*Sample population and setting*

The study will include 700 Mexican cases diagnosed as schizophrenics with or without a suicide attempt and bipolar with or without a suicide attempt; also suicide attempters without psychiatric comorbidity; using Structured Clinical Interview for DSM-IV (SCID-I and II). All patients, will be recruited from three clinical centers ("Dr. Gustavo A. Rovirosa" General Hospital, "Dr. Desiderio G. Carbajal Regional Hospital in Tabasco, Mexico and "Dr. Juan N. Navarro" Psychiatric Hospital in Mexico City). A group of 500 healthy volunteers without any history of psychiatric disorders will be randomly selected as a control group. All individuals will be Mexican with Mexican ancestry at least up to two generations

(Mexican grandparents); they will be recruited from several Mexicans hospitals and outpatient clinics.

*Ethical approval*

A written informed consent will be obtained from all patients who accept to participate. The study will be performed in accordance with the Helsinki declaration (59th General Assembly, Seoul, Korea, October 2008). This study has been approved by the ethics and investigation commitments of the National Institute of Genomic Medicine on July 22nd 2015, No. CEI 215/13

*Clinical assessment*

The diagnoses and clinical evaluations will be performed by at least two trained senior psychiatrists of whom at least one of them would have personally examined the patient. All the subjects included in the study will participate in semi-structured interviews that will include life-time and family history of suicidal behavior, among other clinical features. Subsequently, the sample will be stratified as follows: schizophrenics with and without suicide attempt, bipolar with and without suicide attempt, only suicide attempters and finally, healthy subjects as controls. The suicide attempt will be defined as a self-injurious act that had at least a partial intent to end one's life; the number of attempts, method used and medical damage of past suicide attempts will be recorded.

*Genotyping*

The DNA will be isolated from peripheral blood leukocytes samples using a standardized protocol of the Genomic Wizard Purification Kit from Promega, as

previously reported [22-24]. The integrity of genetic material will be checked on 1% agarose gels and quantified by spectrophotometry using Nanodrop system.

The genotyping will be performed using the Infinium PsychArray BeadChip, following the manufacturer's protocol [25 26]. This array contains approximately 580000 genetic variants, wherein include a set of genetic variants previously associated with a variety of psychiatric illnesses. All genotyping analyses will be performed at the National Institute of Genomic Medicine (INMEGEN). For quality controls, we will filter-out samples and variants with call rates lower than 98% and variants deviating from Hardy-Weinberg equilibrium, with a chi-square p-value < $1x10^{-6}$. The concordance between gender will be performed based on the heterozygosity of the X and Y chromosomes. All the filtering processes will be performed using the PLINK v1.9 software [27]. After the quality control procedures, we will impute variants on autosome chromosomes, with Beagle software [28]; during the imputation process, we will use 1000 genomes phase 3 database as references.

*Statistical analyses*

In order to evaluate the effect of genetic variants on suicidal behavior, we will perform two different workflows: A) a classic GWAS analysis applying the same weight to all the variants and B) an algorithm with prediction of deleteriousness of the variants. Concerning the first workflow, we will conduct a mixed linear model analysis on imputed variants with a minor allele frequency of 5%, adjusting the models with the Genetic Relationship Matrix, age and gender. The implementation of the linear mixed models will be performed on the GCTA software [29]. For the

second workflow, will include a prediction of the deliriousness of the variants using different prediction algorithms, such as PolyPhen, SIFT, CADD, VEP and ENCODE [30-34]. After the prediction of the functional impact of the variants, we will compare the cases allele frequency of loss-of-function, missense with deleterious effect, variants present in regulatory regions and variants with a PHRED-CADD score higher than 20, with the allele frequency of the populations reported in the 1000 genomes and ExAC database.

## Discussion

Various situations and contexts have been proposed as predictors of suicidal behavior; among them, the presence of a psychiatric disorder seems to be an important determinant for such behavior. Likewise, the possible predisposition of a genetic background to manifest SB has been supported by several investigations, but the understanding of the precise genetic system that causes such vulnerability to suicidal tendencies is still largely incomplete. Hence, the principal aim of our protocol study is to explore the potential genetic influence over SB in a Mexican population, throughout a genome-wide association study. In addition, we want to emphasize that to our knowledge, this study protocol will be the first one to evaluate suicidal behavior in schizophrenic and bipolar disorder patients, in a Mexican population.

The majority of the genetic epidemiology evidence suggest that suicidal behavior is a complex issue, where there are multiple genes that have a small effect over SB, but if combined, could become predisposing factors; therefore, association studies that detect small effect contributions can be more useful, which

is a strength of this protocol. In this sense, one of the most powerful strengths of the GWAS is that uses many genetic markers across the whole genome to analyze for associations with a particular disease; as it is based on no prior assumptions, it explores a large number of genetic variants. For these reasons, we will use a GWAS to explore the genetic influence over suicidal behavior in schizophrenics with and without SA, bipolar patients with and without SA, suicide attempters and healthy subjects as controls. Therefore, the results of this study will provide information to better comprehend the influence of the genetic background in the development of suicidal behavior, among psychiatric patients.

Additionally, the findings of the present research could provide valuable information for future researches that attempt to identify genetic risk factors for suicidal behavior, and help detect and/or treat this disease. Nowadays, there are microarrays that have been used to study several genetic variables in Caucasians and Asians; but to our knowledge, there is no evidence reported of GWAS in studies that evaluate suicidal behavior in psychiatric patients. Therefore, the outcomes of the study derived of the current protocol, could provide essential information, because the use of this type of genetic tools will allow us to identify the associated SNPs, missense and insertions and indels in mental illnesses such as schizophrenia and bipolar disorder and their possible participation as predictors of suicidal behavior in a Mexican population. Consequently, these findings could give important information to improve the design of future chips for molecular diagnosis of psychiatric disorders in Mexicans, which will be very useful in the prevention, diagnosis and prognosis of suicidal behavior in Mexico.

In conclusion, the completion of the present protocol could impulse the design of a microarray that includes associated variants to SB in Mexican population. Moreover, the findings will give a better perspective of the participation of the genetic background as a predictor of suicidal behavior in psychiatric diseases. Hence, the outcomes would be useful in genetic research, as well as in prevention and early diagnosis of suicidal behavior in Mexicans.

**Ethics and disseminations:**

The study will be conducted in compliance with local regulations and internationally established principles of the Declaration of Helsinki (59th General Assembly, Seoul, Korea, October 2008). This study has been approved by the ethics and investigation commitments of the National Institute of Genomic Medicine on July 22nd 2015, No. CEI 215/13. Before inclusion, all patients are required to sign an informed consent form

**Consent for publication:**

Not applicable

**Availability of data and material:**

Not applicable

**Competing interests statment:**

The authors declare that they have no competing interests.

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**Author contributions:**

AGM, HN and JJMM conceived the study, participated in its design, helped to draft the manuscript and mentored TBGC. CATZ, AGM and JJMM critically revised successive drafts of the manuscript and provided important intellectual input. CATZ

and TBGC coordinated and supervised the integration of the manuscript. All authors read and approved the final manuscript.

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The protocol study will be a doctoral thesis of TBGC.

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STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

| | Item No | Recommendation | Page |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 1-2 |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 2 |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 4 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 5 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 5 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 5-6 |
| Participants | 6 | (*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls | 6-7 |
| | | (*b*) For matched studies, give matching criteria and the number of controls per case | 6-7 |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 5-6 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | *7-8* |
| Bias | 9 | Describe any efforts to address potential sources of bias | N/A |
| Study size | 10 | Explain how the study size was arrived at | 7-8 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 7 |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 7 |
| | | (*b*) Describe any methods used to examine subgroups and interactions | 7-8 |
| | | (*c*) Explain how missing data were addressed | N/A |
| | | (*d*) If applicable, explain how matching of cases and controls was addressed | N/A |
| | | (*e*) Describe any sensitivity analyses | N/A |
| **Results** | | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | N/A |
| | | (b) Give reasons for non-participation at each stage | N/A |
| | | (c) Consider use of a flow diagram | N/A |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | N/A |
| | | (b) Indicate number of participants with missing data for each variable of interest | N/A |
| Outcome data | 15* | Report numbers in each exposure category, or summary measures of exposure | N/A |

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|---|---|---|---|
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | N/A |
| | | (*b*) Report category boundaries when continuous variables were categorized | N/A |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | N/A |

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| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | N/A |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | N/A |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | N/A |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 9 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 9 |
| **Other information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 10-11 |

*Give information separately for cases and controls.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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# Genome-Wide Association Study of suicide attempt in a Mexican population: A study protocol

Thelma Beatriz González-Castro[1], Alma Delia Genis-Mendoza[2,3], Carlos Alfonso Tovilla-Zarate[4], José Jaime Martínez-Magaña[2], Isela Esther Juarez-Rojop[5], Emmanuel Sarmiento[3], Humberto Nicolini[*2,3]

[1] División Académica Multidisciplinaria de Jalpa de Méndez; Universidad Juárez Autónoma de Tabasco. Jalpa de Méndez, Tabasco, México.
[2] Instituto Nacional de Medicina Genómica (INMEGEN); Secretaria de Salud. Ciudad de México.
[3] Hospital Psiquiátrico Infantil "Dr. Juan N. Navarro". Ciudad de México
[4] División Académica Multidisciplinaria de Comalcalco; Universidad Juárez Autónoma de Tabasco. Comalcalco, Tabasco, México.
[5] División Académica Multidisciplinaria de Ciencias de la Salud; Universidad Juárez Autónoma de Tabasco. Villahermosa, Tabasco, México.
[a] These authors contributed equally to this work

TBGC: thelma.glez.castro@gmail.com; CATZ: alfonso_tovillaz@yahoo.com.mx; JJMM: jimy.10.06@gmail.com; IEJR: iselajuarezrojop@hotmail.com; ES: emmanuelsarmientoh@hotmail.com

TBGC and ADGM contributed equally to this paper

*Correspondence authors: Ph.D. Humberto Nicolini, National Institute of Genomic Medicine (INMEGEN), Psychiatric Care Services (SAP). Periférico Sur 4809, Arenal Tepepan, 14610, Mexico City, Mexico. Phone + 55 5350 1900. Email address: hnicolini@inmegen.gob.mx

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## Abstract

*Introduction*: Suicidality is a complex behavior and a major health problem; the specific features that could predispose to suicidal behavior have been extensively investigated, most frequently in European and Asian populations. Therefore, our aim is to present a protocol that will explore suicide attempt in Mexican individuals diagnosed with psychiatric disorders, by performing a genome-wide association study.

*Method and analysis*: we will perform a GWAS on 700 individuals who have a history of suicide attempt, and compare them to subjects without suicide attempt history diagnostic (n=500). The genotyping will be conducted using the Infinium PsychArray BeadChip and quality controls will be applied to the SNPs genotyped. After that, we will perform the imputation using reference panels provided by the Haplotype Reference Consortium. We will perform two different workflows: A) the classic GWAS analysis applying the same weight to all the variants and B) an algorithm with prediction of deleteriousness of variants.

*Ethics and dissemination*

This study was approved by the ethics and investigation commitments of the National Institute of Genomic Medicine on July 22nd 2015, No. CEI 215/13. We plan to disseminate research findings in scientific conferences and as a manuscript in peer-reviewed journals.

*Trial registration*: CEI 215/13


Keywords: suicide & self-harm; mental health; schizophrenia & psychotic disorders; genetics.

## Article summary

Strengths and limitations of this study

- This will be the first study that aims to perform a genome wide association study to assess suicide attempt in a Mexican population.
- The methodological analysis will explore the hypothesis that there are genes that increase the risk of suicide attempt in Mexican population.
- Recruiting participants from three clinical centers in the country will give a more representative sample of the Mexican population.
- Identifying genetic variants associated with suicide attempt will improve our understanding of the biological underlying this disease and could be useful for future microarrays designed to study Latin American populations.
- As the Mexican population has a heterogeneous genetic background, a possible limitation of this study will be that heterogeneity might interfere in the interpretation of results.

## Introduction

Suicide is one of the leading causes of death worldwide. Individuals diagnosed with a psychiatric disorder have higher rates of suicide compared to the general population, which emphasizes a narrow relationship between suicide and psychiatric disorders. In this line of evidence, a considerable majority of suicide victims have had an undiagnosed psychiatric disorder at the time of death [1-3]. Furthermore, several studies consider psychiatric disorders as one of the main risk factors of suicide. Unfortunately, up to today, the mechanisms that explain this relationship have not been fully disentangled [4 5].

Suicidal behavior (SB), has been defined as a complex issue that results from the combination of genetic variants along with personal experiences and environmental contribution; altogether, these factors establish the disease symptomatology manifestation [1, 3, 12]. The results of studies based on twins, adoptions and families, support the heritability of SB, pinpointing a genetic influence [6-9]. Subsequently, many candidate-gene association studies have been performed, with the different phenotypes of SB: suicide attempts, suicide ideation and accomplished suicide [1 3 10-12]. Unfortunately, SB is a polygenic trait and candidate-gene association studies underestimate the genetic background. In an attempt to search for this polygenic variation of SB, other strategies have been developed, such as the genome-wide association studies (GWAS) [12-14].

Over the past decade, a small number of GWAS exploring common genetic variation mostly in suicide attempters, have found significant associations between genetic components and SB, establishing possible molecular pathways involved in the susceptibility of the disease [15 16]. Although a genetic diathesis model for

predisposition to SB has been proposed and GWAS have suggested candidate loci or pathways, the only information available comes from studies analyzing Caucasians or Asians populations, leaving behind Latin American populations including Mexicans [17-21]. Therefore, more studies are necessary in order to have a better comprehension of the genetic background of SB.

Objective

Our aim is to perform the first genome-wide association study of suicide attempters in a Mexican population, in order to explore and define the involvement of a genetic diathesis that predisposes to SB in this population. In addition, we will explore the hypothesis that there are genes and genetic variants that increase the risk of suicide attempt in Mexican population and these factors could be common in individuals with a psychiatric diagnostic.

**Methods and analysis**

*Sample population and setting*

The case group (n=700) will be formed by individuals who have had at least one suicide attempt, and this will be determined using Structured Clinical Interview for DSM-IV (SCID-I and II) in the Psychiatry out-patient area from three clinical centers: “Dr. Gustavo A. Rovirosa” General Hospital in Tabasco, “Dr. Desiderio G. Carbajal Regional Hospital in Tabasco, Mexico and “Dr. Juan N. Navarro” Psychiatric Hospital in Mexico City. Individuals used as controls (n=500) will not have current or past history of any suicidal behavior, with no first degree relatives with a history of suicidal behavior and they will have to be unrelated to those in the

case group. Both cases and controls will be interviewed by two psychiatrist or clinical specialists, together will determine the presence or absence of suicide attempt. All individuals will be Mexican with Mexican ascendancy of at least two generations (Mexican parents and grandparents); they will be recruited from several Mexicans hospitals and outpatient clinics.

*Patient and Public Involvement*

The outcomes of the present protocol will be directly communicated to the patients who participate or their legal caregivers. The results will also be discussed with their psychiatrists and the corresponding health-education structures of the clinical centers. However, the patients have not been directly participate in the study design or any methodological procedures. Nevertheless, the authors of this protocol have clinical experience with SB patients and their families, which helped designed this study.

*Ethics and dissemination*

A written informed consent will be obtained from all individuals who accept to participate. The study will be performed in accordance with the Helsinki declaration (59th General Assembly, Seoul, Korea, October 2008). This study was approved by the ethics and investigation commitments of the National Institute of Genomic Medicine on July 22nd 2015, No. CEI 215/13. We plan to disseminate research findings through scientific conferences and as a manuscript in peer-reviewed journals.

*Clinical assessment*

Diagnoses and clinical evaluations will be performed by at least two trained senior psychiatrists of whom at least one of them would have personally examined the patient. All participants will undergo semi-structured interviews that will include life-time and family history of suicidal behavior, among other clinical features. Suicide attempt will be defined as a self-injurious act that had at least a partial intent to end one's life; the number of attempts, method used and medical damage of past suicide attempts will be obtained.

*Genotyping*

The DNA will be isolated from peripheral blood leukocytes samples using a standardized protocol of the Genomic Wizard Purification Kit from Promega, as previously reported [22-24]. The integrity of genetic material will be checked on 1% agarose gels and quantified by spectrophotometry using Nanodrop system.

The genotyping will be performed using the Infinium PsychArray BeadChip, following the manufacturer's protocol [25 26]. This array contains approximately 580000 genetic variants, wherein include a set of genetic variants previously associated with a variety of psychiatric illnesses. All genotyping analyses will be performed at the National Institute of Genomic Medicine (INMEGEN). For quality controls, we will filter-out samples and variants with call rates lower than 98% and variants deviating from Hardy-Weinberg equilibrium, with a chi-square p-value < $1x10^{-6}$. The concordance between gender will be performed based on the heterozygosity of the X and Y chromosomes. All the filtering processes will be performed using the PLINK v1.9 software [27]. After that, we will perform a

multidimensional scaling (MDS) analysis in PLINK, all SNPs that pass quality controls will be pruned and used to check population stratification in order to evaluate the ancestry of individuals included. After the MDS analysis, the first five components will be used as covariates in the association analysis. MDS dimensions will be graphically represented using the "MDS-plot" option. After quality control procedures, we will perform the imputation using the reference panels provided by the Haplotype Reference Consortium r.1.1; we will use the Michigan Imputation Server for the imputation [28 29].

*Polygenic risk score calculation (PRS)*

PRS is a measurement of genetic liability to schizophrenia based on the Psychiatric Genomics Consortium (PGC) schizophrenia GWAS[30]. SNPs will be selected to be included in the PRS calculation based on p values obtained in the original PGC GWAS using PRSice[31]. PRS will be used to test an association with suicide attempt using linear regression models adjusted by age, sex, and four multidimensional-scaling components (MDS). The estimation of gene- or set-based association tests using GWAS summary data, will be performed using Genome-wide Complex Trait Analysis (GCTA) [32 33].

*Statistical analyses*

In order to evaluate the effect of genetic variants on suicidal behavior, we will perform two different workflows: A) a classic GWAS analysis applying the same importance to all the variants and B) an algorithm with prediction of deleteriousness of the variants. Concerning the first workflow, we will conduct a

mixed linear model analysis on imputed variants with a minor allele frequency of 5%. The implementation of linear mixed models will be performed on the GCTA software [34]. For the second workflow, will include a prediction of the deliriousness of variants using different prediction algorithms, such as PolyPhen, SIFT, CADD, VEP and ENCODE [35-39]. After the prediction of the functional impact of the variants, we will compare the cases allele frequency of loss-of-function, missense with deleterious effect, variants present in regulatory regions and variants with a PHRED-CADD score higher than 20, with the allele frequency of the populations reported in the 1000 genomes and ExAC database. GWAS analysis will be performed on cases with a history of suicide attempts and compared to non-suicide attempters. For the joint analysis, we will investigate the possibility of a new association within the genome; therefore, multiple testing corrections will be conducted using the Bonferroni correction and permutation; the corrected p value will depend on the total number of independent tests.

*Power analysis calculation*

For the primary analysis of the GWAS suicide attempt using 700 cases and 500 controls, we will use a log additive model of inheritance, MAF of 25%, $P_0$ of 0.08 the power will be 0.99. All the calculations will be performed using QUANTO 1.2.4. (http://biostats.usc.edu/software), as previously reported[14].

## Discussion

Various situations and contexts have been proposed as predictors of suicidal behavior; among them, the presence of a psychiatric disorder seems to be

an important determinant for such behavior. Likewise, the possible predisposition of a genetic background to manifest SB has been supported by several investigations, but the understanding of the precise genetic system that causes such vulnerability to suicidal tendencies is still largely incomplete. Hence, the principal aim of our protocol study is to explore the potential genetic influence on SB in a Mexican population, throughout a genome-wide association study. In addition, we want to emphasize that to our knowledge, this study protocol will be the first one to evaluate suicidal behavior in patients with schizophrenia and bipolar disorder in a Mexican population.

The majority of the genetic epidemiology evidence suggest that suicidal behavior is a complex issue, where there are multiple genes that have a small effect over SB; but if combined, could become predisposing factors. Therefore, association studies that detect small effect contributions can be more useful, which is a strength of this protocol. In this sense, one of the most powerful strengths of the GWAS is that it uses many genetic markers across the whole genome to search for associations with a particular disease; as it is based on no prior assumptions, it explores a large number of genetic variants. For these reasons, we will use GWAS to explore the genetic influence on suicidal behavior in schizophrenics with and without SA, bipolar patients with and without SA, suicide attempters and healthy subjects as controls. Therefore, the results of this study will provide information to better comprehend the influence of the genetic background on the development of suicidal behavior, among psychiatric patients.

Additionally, the findings of the present research could provide valuable information for future researches who attempt to identify genetic risk factors of suicidal

behavior, and help detect and/or treat this disease. Nowadays, there are microarrays that have been used to study several genetic variables in Caucasian and Asian populations; but to our knowledge, there is no evidence reported of GWAS in studies that evaluate suicidal behavior in psychiatric patients. Therefore, the outcomes of the study derived of the current protocol, could provide essential information. The use of this type of genetic tools will allow us to identify associated SNPs, missense and insertions and indels in mental illnesses such as schizophrenia and bipolar disorder, as well as their possible participation as predictors of suicidal behavior in a Mexican population. Consequently, these findings could give important information to improve the design of future chips for molecular diagnosis of psychiatric disorders in Mexicans, which will be very useful in the prevention, diagnosis and prognosis of suicidal behavior in Mexico.

In conclusion, the completion of the present protocol could impulse the design of a microarray that includes associated variants to SB in Mexican population. Moreover, the findings will give a better perspective of the participation of the genetic background as a predictor of suicidal behavior in psychiatric diseases. Hence, the outcomes would be useful in genetic research, as well as in prevention and early diagnosis of suicidal behavior in Mexicans

**Ethics and disseminations:**

The study will be conducted in compliance with local regulations and internationally established principles of the Declaration of Helsinki (59th General Assembly, Seoul, Korea, October 2008). This study was approved by the ethics and investigation commitments of the National Institute of Genomic Medicine on July 22nd 2015, No. CEI 215/13. Before inclusion, all patients are required to sign an informed consent form.

**Consent for publication:**

Not applicable.

**Availability of data and material:**

Not applicable.

**Competing interest statement:**

The authors declare to have no competing interests.

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**Author contributions:**

AGM, HN and JJMM conceived the study, participated in its design, helped to draft the manuscript and mentored TBGC. CATZ, AGM and JJMM critically revised successive drafts of the manuscript and provided important intellectual input. CATZ and TBGC coordinated and supervised the integration of the manuscript. All authors read and approved the final manuscript.

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The protocol study will be a doctoral thesis of TBGC.

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Thelma Beatriz González-Castro[1], Alma Delia Genis-Mendoza[2,3], Carlos Alfonso Tovilla-Zarate[4], José Jaime Martínez-Magaña[2], Isela Esther Juarez-Rojop[5], Emmanuel Sarmiento[3], Humberto Nicolini[*2,3]

[1] División Académica Multidisciplinaria de Jalpa de Méndez; Universidad Juárez Autónoma de Tabasco. Jalpa de Méndez, Tabasco, México.
[2] Instituto Nacional de Medicina Genómica (INMEGEN); Secretaria de Salud. Ciudad de México.
[3] Hospital Psiquiátrico Infantil "Dr. Juan N. Navarro". Ciudad de México
[4] División Académica Multidisciplinaria de Comalcalco; Universidad Juárez Autónoma de Tabasco. Comalcalco, Tabasco, México.
[5] División Académica Multidisciplinaria de Ciencias de la Salud; Universidad Juárez Autónoma de Tabasco. Villahermosa, Tabasco, México.
[a] These authors contributed equally to this work

TBGC: thelma.glez.castro@gmail.com; CATZ: alfonso_tovillaz@yahoo.com.mx; JJMM: jimy.10.06@gmail.com; IEJR: iselajuarezrojop@hotmail.com; ES: emmanuelsarmientoh@hotmail.com

TBGC and ADGM contributed equally to this paper

*Correspondence authors: Ph.D. Humberto Nicolini, National Institute of Genomic Medicine (INMEGEN), Psychiatric Care Services (SAP). Periférico Sur 4809, Arenal Tepepan, 14610, Mexico City, Mexico. Phone + 55 5350 1900. Email address: hnicolini@inmegen.gob.mx

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Strengths and limitations of this study

- This will be the first study that aims to perform a genome wide association study to assess suicide attempt in a Mexican population.
- The methodological analysis will explore the hypothesis that there are genes that increase the risk of suicide attempt in Mexican population.
- Recruiting participants from three clinical centers in the country will give a more representative sample of the Mexican population.
- Identifying genetic variants associated with suicide attempt will improve our understanding of the biological underlying this disease and could be useful for future microarrays designed to study Latin American populations.
- As the Mexican population has a heterogeneous genetic background, a possible limitation of this study will be that heterogeneity might interfere in the interpretation of results.

## Introduction

Suicide is one of the leading causes of death worldwide. Individuals diagnosed with a psychiatric disorder have higher rates of suicide compared to the general population, which emphasizes a narrow relationship between suicide and psychiatric disorders. In this line of evidence, a considerable majority of suicide victims have had an undiagnosed psychiatric disorder at the time of death [1-3]. Furthermore, several studies consider psychiatric disorders as one of the main risk factors of suicide. Unfortunately, up to today, the mechanisms that explain this relationship have not been fully disentangled [4 5].

Suicidal behavior (SB), has been defined as a complex issue that results from the combination of genetic variants along with personal experiences and environmental contribution; altogether, these factors establish the disease symptomatology manifestation [1, 3, 12]. The results of studies based on twins, adoptions and families, support the heritability of SB, pinpointing a genetic influence [6-9]. Subsequently, many candidate-gene association studies have been performed, with the different phenotypes of SB: suicide attempts, suicide ideation and accomplished suicide [1 3 10-12]. Unfortunately, SB is a polygenic trait and candidate-gene association studies underestimate the genetic background. In an attempt to search for this polygenic variation of SB, other strategies have been developed, such as the genome-wide association studies (GWAS) [12-14].

Over the past decade, a small number of GWAS exploring common genetic variation mostly in suicide attempters, have found significant associations between genetic components and SB, establishing possible molecular pathways involved in the susceptibility of the disease [15 16]. Although a genetic diathesis model for

predisposition to SB has been proposed and GWAS have suggested candidate loci or pathways, the only information available comes from studies analyzing Caucasians or Asians populations, leaving behind Latin American populations including Mexicans [17-21]. Therefore, more studies are necessary in order to have a better comprehension of the genetic background of SB.

Objective

Our aim is to perform the first genome-wide association study of suicide attempters in a Mexican population, in order to explore and define the involvement of a genetic diathesis that predisposes to SB in this population. In addition, we will explore the hypothesis that there are genes and genetic variants that increase the risk of suicide attempt in Mexican population and these factors could be common in individuals with a psychiatric diagnostic.

**Methods and analysis**

*Sample population and setting*

The case group (n=700) will be formed by individuals who have had at least one suicide attempt, and this will be determined using Structured Clinical Interview for DSM-IV (SCID-I and II) in the Psychiatry out-patient area from three clinical centers: “Dr. Gustavo A. Rovirosa” General Hospital in Tabasco, “Dr. Desiderio G. Carbajal Regional Hospital in Tabasco, Mexico and “Dr. Juan N. Navarro” Psychiatric Hospital in Mexico City. Individuals used as controls (n=500) will not have current or past history of any suicidal behavior, with no first degree relatives with a history of suicidal behavior and they will have to be unrelated to those in the

case group. Both cases and controls will be interviewed by two psychiatrist or clinical specialists, together will determine the presence or absence of suicide attempt. All individuals will be Mexican with Mexican ascendancy of at least two generations (Mexican parents and grandparents); they will be recruited from several Mexicans hospitals and outpatient clinics.

*Patient and Public Involvement*

The outcomes of the present protocol will be directly communicated to the patients who participate or their legal caregivers. The results will also be discussed with their psychiatrists and the corresponding health-education structures of the clinical centers. However, the patients have not been directly participate in the study design or any methodological procedures. Nevertheless, the authors of this protocol have clinical experience with SB patients and their families, which helped designed this study.

*Ethics and dissemination*

A written informed consent will be obtained from all individuals who accept to participate. The study will be performed in accordance with the Helsinki declaration (59th General Assembly, Seoul, Korea, October 2008). This study was approved by the ethics and investigation commitments of the National Institute of Genomic Medicine on July 22nd 2015, No. CEI 215/13. We plan to disseminate research findings through scientific conferences and as a manuscript in peer-reviewed journals.

*Clinical assessment*

Diagnoses and clinical evaluations will be performed by at least two trained senior psychiatrists of whom at least one of them would have personally examined the patient. All participants will undergo semi-structured interviews that will include life-time and family history of suicidal behavior, among other clinical features. Suicide attempt will be defined as a self-injurious act that had at least a partial intent to end one's life; the number of attempts, method used and medical damage of past suicide attempts will be obtained.

*Genotyping*

The DNA will be isolated from peripheral blood leukocytes samples using a standardized protocol of the Genomic Wizard Purification Kit from Promega, as previously reported [22-24]. The integrity of genetic material will be checked on 1% agarose gels and quantified by spectrophotometry using Nanodrop system.

The genotyping will be performed using the Infinium PsychArray BeadChip, following the manufacturer's protocol [25 26]. This array contains approximately 580000 genetic variants, wherein include a set of genetic variants previously associated with a variety of psychiatric illnesses. All genotyping analyses will be performed at the National Institute of Genomic Medicine (INMEGEN). For quality controls, we will filter-out samples and variants with call rates lower than 98% and variants deviating from Hardy-Weinberg equilibrium, with a chi-square p-value < $1x10^{-6}$. The concordance between gender will be performed based on the heterozygosity of the X and Y chromosomes. All the filtering processes will be performed using the PLINK v1.9 software [27]. After that, we will perform a

multidimensional scaling (MDS) analysis in PLINK, all SNPs that pass quality controls will be pruned and used to check population stratification in order to evaluate the ancestry of individuals included. After the MDS analysis, the first five components will be used as covariates in the association analysis. MDS dimensions will be graphically represented using the "MDS-plot" option. After quality control procedures, we will perform the imputation using the reference panels provided by the Haplotype Reference Consortium r.1.1; we will use the Michigan Imputation Server for the imputation [28 29].

*Polygenic risk score calculation (PRS)*

PRS is a measurement of genetic liability to schizophrenia based on the Psychiatric Genomics Consortium (PGC) schizophrenia GWAS[30]. SNPs will be selected to be included in the PRS calculation based on p values obtained in the original PGC GWAS using PRSice[31]. PRS will be used to test an association with suicide attempt using linear regression models adjusted by age, sex, and four multidimensional-scaling components (MDS). The estimation of gene- or set-based association tests using GWAS summary data, will be performed using Genome-wide Complex Trait Analysis (GCTA) [32 33].

*Statistical analyses*

In order to evaluate the effect of genetic variants on suicidal behavior, we will perform two different workflows: A) a classic GWAS analysis applying the same importance to all the variants and B) an algorithm with prediction of deleteriousness of the variants. Concerning the first workflow, we will conduct a

mixed linear model analysis on imputed variants with a minor allele frequency of 5%. The implementation of linear mixed models will be performed on the GCTA software [34]. For the second workflow, will include a prediction of the deliriousness of variants using different prediction algorithms, such as PolyPhen, SIFT, CADD, VEP and ENCODE [35-39]. After the prediction of the functional impact of the variants, we will compare the cases allele frequency of loss-of-function, missense with deleterious effect, variants present in regulatory regions and variants with a PHRED-CADD score higher than 20, with the allele frequency of the populations reported in the 1000 genomes and ExAC database. GWAS analysis will be performed on cases with a history of suicide attempts and compared to non-suicide attempters. Genome wide significance will be set at $P < 5x10^{-08}$ .

*Power analysis calculation*

For GWAS analysis, we performed power calculations in QUANTO 1.2.4 (http://biostats.usc.edu/software) [14]. The analysis use a log additive model of inheritance to detect a power of 0.98 at significance threshold $5x10^{-08}$ to detect an effect size of OR ≥ 2 with a MAF of 0.25 and a $P_0$ of 0.08. Also testing the lower bounds of the effect sizes of the variants, we observe a power of 0.82 with an effect size of 1.8, $P_0$=0.08 and MAF=0.25 at the same significance level. The $P_0$ that we used is the baseline risk of suicide attempt in our population based on previous reports [40 41]. Therefore, our study will be powered to detect genetics effects.

## Discussion

Various situations and contexts have been proposed as predictors of suicidal behavior; among them, the presence of a psychiatric disorder seems to be an important determinant for such behavior. Likewise, the possible predisposition of a genetic background to manifest SB has been supported by several investigations, but the understanding of the precise genetic system that causes such vulnerability to suicidal tendencies is still largely incomplete. Hence, the principal aim of our protocol study is to explore the potential genetic influence on SB in a Mexican population, throughout a genome-wide association study. In addition, we want to emphasize that to our knowledge, this study protocol will be the first one to evaluate suicidal behavior in patients with schizophrenia and bipolar disorder in a Mexican population.

The majority of the genetic epidemiology evidence suggest that suicidal behavior is a complex issue, where there are multiple genes that have a small effect over SB; but if combined, could become predisposing factors. Therefore, association studies that detect small effect contributions can be more useful, which is a strength of this protocol. In this sense, one of the most powerful strengths of the GWAS is that it uses many genetic markers across the whole genome to search for associations with a particular disease; as it is based on no prior assumptions, it explores a large number of genetic variants. For these reasons, we will use GWAS to explore the genetic influence on suicidal behavior in schizophrenics with and without SA, bipolar patients with and without SA, suicide attempters and healthy subjects as controls. Therefore, the results of this study will

provide information to better comprehend the influence of the genetic background on the development of suicidal behavior, among psychiatric patients.

Additionally, the findings of the present research could provide valuable information for future researches who attempt to identify genetic risk factors of suicidal behavior, and help detect and/or treat this disease. Nowadays, there are microarrays that have been used to study several genetic variables in Caucasian and Asian populations; but to our knowledge, there is no evidence reported of GWAS in studies that evaluate suicidal behavior in psychiatric patients. Therefore, the outcomes of the study derived of the current protocol, could provide essential information. The use of this type of genetic tools will allow us to identify associated SNPs, missense and insertions and indels in mental illnesses such as schizophrenia and bipolar disorder, as well as their possible participation as predictors of suicidal behavior in a Mexican population. Consequently, these findings could give important information to improve the design of future chips for molecular diagnosis of psychiatric disorders in Mexicans, which will be very useful in the prevention, diagnosis and prognosis of suicidal behavior in Mexico.

In conclusion, the completion of the present protocol could impulse the design of a microarray that includes associated variants to SB in Mexican population. Moreover, the findings will give a better perspective of the participation of the genetic background as a predictor of suicidal behavior in psychiatric diseases. Hence, the outcomes would be useful in genetic research, as well as in prevention and early diagnosis of suicidal behavior in Mexicans

**Ethics and disseminations:**

The study will be conducted in compliance with local regulations and internationally established principles of the Declaration of Helsinki (59th General Assembly, Seoul, Korea, October 2008). This study was approved by the ethics and investigation commitments of the National Institute of Genomic Medicine on July 22nd 2015, No. CEI 215/13. Before inclusion, all patients are required to sign an informed consent form.

**Consent for publication:**

Not applicable.

**Availability of data and material:**

Not applicable.

**Competing interest statement:**

The authors declare to have no competing interests.

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**Author contributions:**

ADGM, HN and JJMM conceived the study, participated in its design, helped to draft the manuscript and mentored TBGC. CATZ, ADGM and JJMM critically revised successive drafts of the manuscript and provided important intellectual input. CATZ and TBGC coordinated and supervised the integration of the manuscript. ES, IEJR, HN and ADGM contributed to developing the analytic plan proposed for this study. All authors read and approved the final manuscript.

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The protocol study will be a doctoral thesis of TBGC.

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# Genome-Wide Association Study of suicide attempt in a Mexican population: A study protocol

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# Genome-Wide Association Study of suicide attempt in a Mexican population: A study protocol

Thelma Beatriz González-Castro[1], Alma Delia Genis-Mendoza[2,3], Carlos Alfonso Tovilla-Zarate[4], José Jaime Martínez-Magaña[2], Isela Esther Juarez-Rojop[5], Emmanuel Sarmiento[3], Humberto Nicolini[*2,3]

[1] División Académica Multidisciplinaria de Jalpa de Méndez; Universidad Juárez Autónoma de Tabasco. Jalpa de Méndez, Tabasco, México.
[2] Instituto Nacional de Medicina Genómica (INMEGEN); Secretaria de Salud. Ciudad de México.
[3] Hospital Psiquiátrico Infantil "Dr. Juan N. Navarro". Ciudad de México
[4] División Académica Multidisciplinaria de Comalcalco; Universidad Juárez Autónoma de Tabasco. Comalcalco, Tabasco, México.
[5] División Académica Multidisciplinaria de Ciencias de la Salud; Universidad Juárez Autónoma de Tabasco. Villahermosa, Tabasco, México.
[a] These authors contributed equally to this work

TBGC: thelma.glez.castro@gmail.com; CATZ: alfonso_tovillaz@yahoo.com.mx; JJMM: jimy.10.06@gmail.com; IEJR: iselajuarezrojop@hotmail.com; ES: emmanuelsarmientoh@hotmail.com

TBGC and ADGM contributed equally to this paper

*Correspondence authors: Ph.D. Humberto Nicolini, National Institute of Genomic Medicine (INMEGEN), Psychiatric Care Services (SAP). Periférico Sur 4809, Arenal Tepepan, 14610, Mexico City, Mexico. Phone + 55 5350 1900. Email address: hnicolini@inmegen.gob.mx

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## Abstract

*Introduction*: Suicidality is a complex behavior and a major health problem; the specific features that could predispose to suicidal behavior have been extensively investigated, most frequently in European and Asian populations. Therefore, our aim is to present a protocol that will explore suicide attempt in Mexican individuals diagnosed with psychiatric disorders, by performing a genome-wide association study.

*Method and analysis*: we will perform a GWAS on 700 individuals who have a history of suicide attempt, and compare them to subjects without suicide attempt history diagnostic (n=500). The genotyping will be conducted using the Infinium PsychArray BeadChip and quality controls will be applied to the SNPs genotyped. After that, we will perform the imputation using reference panels provided by the Haplotype Reference Consortium. We will perform two different workflows: A) the classic GWAS analysis applying the same weight to all the variants and B) an algorithm with prediction of deleteriousness of variants.

*Ethics and dissemination*

This study was approved by the ethics and investigation commitments of the National Institute of Genomic Medicine on July 22nd 2015, No. CEI 215/13. We plan to disseminate research findings in scientific conferences and as a manuscript in peer-reviewed journals.

*Trial registration*: CEI 215/13


Keywords: suicide & self-harm; mental health; schizophrenia & psychotic disorders; genetics.

## Article summary

Strengths and limitations of this study

- This will be the first study that aims to perform a genome wide association study to assess suicide attempt in a Mexican population.
- The methodological analysis will explore the hypothesis that there are genes that increase the risk of suicide attempt in Mexican population.
- Recruiting participants from three clinical centers in the country will give a more representative sample of the Mexican population.
- Identifying genetic variants associated with suicide attempt will improve our understanding of the biological underlying this disease and could be useful for future microarrays designed to study Latin American populations.
- As the Mexican population has a heterogeneous genetic background, a possible limitation of this study will be that heterogeneity might interfere in the interpretation of results.

## Introduction

Suicide is one of the leading causes of death worldwide. Individuals diagnosed with a psychiatric disorder have higher rates of suicide compared to the general population, which emphasizes a narrow relationship between suicide and psychiatric disorders. In this line of evidence, a considerable majority of suicide victims have had an undiagnosed psychiatric disorder at the time of death[1-3]. Furthermore, several studies consider psychiatric disorders as one of the main risk factors of suicide. Unfortunately, up to today, the mechanisms that explain this relationship have not been fully disentangled[4 5].

Suicidal behavior (SB), has been defined as a complex issue that results from the combination of genetic variants along with personal experiences and environmental contribution; altogether, these factors establish the disease symptomatology manifestation [1 3 6]. The results of studies based on twins, adoptions and families, support the heritability of SB, pinpointing a genetic influence[7-10]. Subsequently, many candidate-gene association studies have been performed, with the different phenotypes of SB: suicide attempts, suicide ideation and accomplished suicide [1 3 6 11 12]. Unfortunately, SB is a polygenic trait and candidate-gene association studies underestimate the genetic background. In an attempt to search for this polygenic variation of SB, other strategies have been developed, such as the genome-wide association studies (GWAS) [6 13 14].

Over the past decade, a small number of GWAS exploring common genetic variation mostly in suicide attempters, have found significant associations between genetic components and SB, establishing possible molecular pathways involved in the susceptibility of the disease[15 16]. Although a genetic diathesis model for

predisposition to SB has been proposed and GWAS have suggested candidate loci or pathways, the only information available comes from studies analyzing Caucasians or Asians populations, leaving behind Latin American populations including Mexicans[17-21]. Therefore, more studies are necessary in order to have a better comprehension of the genetic background of SB.

Objective

Our aim is to perform the first genome-wide association study of suicide attempters in a Mexican population, in order to explore and define the involvement of a genetic diathesis that predisposes to SB in this population. In addition, we will explore the hypothesis that there are genes and genetic variants that increase the risk of suicide attempt in Mexican population and these factors could be common in individuals with a psychiatric diagnostic.

**Methods and analysis**

*Sample population and setting*

The case group (n=700) will be formed by individuals who have had at least one suicide attempt, and this will be determined using Structured Clinical Interview for DSM-IV (SCID-I and II) in the Psychiatry out-patient area from three clinical centers: “Dr. Gustavo A. Rovirosa” General Hospital in Tabasco, “Dr. Desiderio G. Carbajal Regional Hospital in Tabasco, Mexico and “Dr. Juan N. Navarro” Psychiatric Hospital in Mexico City. Individuals used as controls (n=500) will not have current or past history of any suicidal behavior, with no first degree relatives with a history of suicidal behavior and they will have to be unrelated to those in the

case group. Both cases and controls will be interviewed by two psychiatrist or clinical specialists, together will determine the presence or absence of suicide attempt. All individuals will be Mexican with Mexican ascendancy of at least two generations (Mexican parents and grandparents); they will be recruited from several Mexicans hospitals and outpatient clinics.

*Patient and Public Involvement*

The outcomes of the present protocol will be directly communicated to the patients who participate or their legal caregivers. The results will also be discussed with their psychiatrists and the corresponding health-education structures of the clinical centers. However, the patients have not been directly participate in the study design or any methodological procedures. Nevertheless, the authors of this protocol have clinical experience with SB patients and their families, which helped designed this study.

*Ethics and dissemination*

A written informed consent will be obtained from all individuals who accept to participate. The study will be performed in accordance with the Helsinki declaration (59th General Assembly, Seoul, Korea, October 2008). This study was approved by the ethics and investigation commitments of the National Institute of Genomic Medicine on July 22nd 2015, No. CEI 215/13. We plan to disseminate research findings through scientific conferences and as a manuscript in peer-reviewed journals.

*Clinical assessment*

Diagnoses and clinical evaluations will be performed by at least two trained senior psychiatrists of whom at least one of them would have personally examined the patient. All participants will undergo semi-structured interviews that will include life-time and family history of suicidal behavior, among other clinical features. Suicide attempt will be defined as a self-injurious act that had at least a partial intent to end one's life; the number of attempts, method used and medical damage of past suicide attempts will be obtained.

*Genotyping*

The DNA will be isolated from peripheral blood leukocytes samples using a standardized protocol of the Genomic Wizard Purification Kit from Promega, as previously reported[22-24]. The integrity of genetic material will be checked on 1% agarose gels and quantified by spectrophotometry using Nanodrop system.

The genotyping will be performed using the Infinium PsychArray BeadChip, following the manufacturer's protocol [25 26]. This array contains approximately 580000 genetic variants, wherein include a set of genetic variants previously associated with a variety of psychiatric illnesses. All genotyping analyses will be performed at the National Institute of Genomic Medicine (INMEGEN). For quality controls, we will filter-out samples and variants with call rates lower than 98% and variants deviating from Hardy-Weinberg equilibrium, with a chi-square p-value < $1x10^{-6}$. The concordance between gender will be performed based on the heterozygosity of the X and Y chromosomes. All the filtering processes will be performed using the PLINK v1.9 software [27]. After that, we will perform a

multidimensional scaling (MDS) analysis in PLINK, all SNPs that pass quality controls will be pruned and used to check population stratification in order to evaluate the ancestry of individuals included. After the MDS analysis, the first five components will be used as covariates in the association analysis. MDS dimensions will be graphically represented using the "MDS-plot" option. After quality control procedures, we will perform the imputation using the reference panels provided by the Haplotype Reference Consortium r.1.1; we will use the Michigan Imputation Server for the imputation [28 29].

*Polygenic risk score calculation (PRS)*

PRS is a measurement of genetic liability to schizophrenia based on the Psychiatric Genomics Consortium (PGC) schizophrenia GWAS[30]. SNPs will be selected to be included in the PRS calculation based on p values obtained in the original PGC GWAS using PRSice[31]. PRS will be used to test an association with suicide attempt using linear regression models adjusted by age, sex, and four multidimensional-scaling components (MDS). The estimation of gene- or set-based association tests using GWAS summary data, will be performed using Genome-wide Complex Trait Analysis (GCTA) [32 33].

*Statistical analyses*

In order to evaluate the effect of genetic variants on suicidal behavior, we will perform two different workflows: A) a classic GWAS analysis applying the same importance to all the variants and B) an algorithm with prediction of deleteriousness of the variants. Concerning the first workflow, we will conduct a

mixed linear model analysis on imputed variants with a minor allele frequency of 5%. The implementation of linear mixed models will be performed on the GCTA software [34]. For the second workflow, will include a prediction of the deliriousness of variants using different prediction algorithms, such as PolyPhen, SIFT, CADD, VEP and ENCODE [35-39]. After the prediction of the functional impact of the variants, we will compare the cases allele frequency of loss-of-function, missense with deleterious effect, variants present in regulatory regions and variants with a PHRED-CADD score higher than 20, with the allele frequency of the populations reported in the 1000 genomes and ExAC database. GWAS analysis will be performed on cases with a history of suicide attempts and compared to non-suicide attempters. Genome wide significance will be set at $P < 5x10^{-08}$ .

*Power analysis calculation*

For GWAS analysis, we performed power calculations in QUANTO 1.2.4 (http://biostats.usc.edu/software). The analysis uses a log additive model of inheritance to detect a power of 0.82 at significance threshold $5x10^{-08}$ to detect an effect size of OR ≥ 1.8 with a $P_0$ of 0.08 and MAF of 0.25, as in a previous study[14]. The $P_0$ that we used is the baseline risk of suicide attempt in our population based on previous reports [40 41]. Therefore, our study will be powered to detect genetics effects.

## Discussion

Various situations and contexts have been proposed as predictors of suicidal behavior; among them, the presence of a psychiatric disorder seems to be an important determinant for such behavior. Likewise, the possible predisposition of a genetic background to manifest SB has been supported by several investigations, but the understanding of the precise genetic system that causes such vulnerability to suicidal tendencies is still largely incomplete. Hence, the principal aim of our protocol study is to explore the potential genetic influence on SB in a Mexican population, throughout a genome-wide association study. In addition, we want to emphasize that to our knowledge, this study protocol will be the first one to evaluate suicidal behavior in patients with schizophrenia and bipolar disorder in a Mexican population.

The majority of the genetic epidemiology evidence suggest that suicidal behavior is a complex issue, where there are multiple genes that have a small effect over SB; but if combined, could become predisposing factors. Therefore, association studies that detect small effect contributions can be more useful, which is a strength of this protocol. In this sense, one of the most powerful strengths of the GWAS is that it uses many genetic markers across the whole genome to search for associations with a particular disease; as it is based on no prior assumptions, it explores a large number of genetic variants. For these reasons, we will use GWAS to explore the genetic influence on suicidal behavior in schizophrenics with and without SA, bipolar patients with and without SA, suicide attempters and healthy subjects as controls. Therefore, the results of this study will

provide information to better comprehend the influence of the genetic background on the development of suicidal behavior, among psychiatric patients.

Additionally, the findings of the present research could provide valuable information for future researches who attempt to identify genetic risk factors of suicidal behavior, and help detect and/or treat this disease. Nowadays, there are microarrays that have been used to study several genetic variables in Caucasian and Asian populations; but to our knowledge, there is no evidence reported of GWAS in studies that evaluate suicidal behavior in psychiatric patients. Therefore, the outcomes of the study derived of the current protocol, could provide essential information. The use of this type of genetic tools will allow us to identify associated SNPs, missense and insertions and indels in mental illnesses such as schizophrenia and bipolar disorder, as well as their possible participation as predictors of suicidal behavior in a Mexican population. Consequently, these findings could give important information to improve the design of future chips for molecular diagnosis of psychiatric disorders in Mexicans, which will be very useful in the prevention, diagnosis and prognosis of suicidal behavior in Mexico.

In conclusion, the completion of the present protocol could impulse the design of a microarray that includes associated variants to SB in Mexican population. Moreover, the findings will give a better perspective of the participation of the genetic background as a predictor of suicidal behavior in psychiatric diseases. Hence, the outcomes would be useful in genetic research, as well as in prevention and early diagnosis of suicidal behavior in Mexicans

**Ethics and disseminations:**

The study will be conducted in compliance with local regulations and internationally established principles of the Declaration of Helsinki (59th General Assembly, Seoul, Korea, October 2008). This study was approved by the ethics and investigation commitments of the National Institute of Genomic Medicine on July 22nd 2015, No. CEI 215/13. Before inclusion, all patients are required to sign an informed consent form.

**Consent for publication:**

Not applicable.

**Availability of data and material:**

Not applicable.

**Competing interest statement:**

The authors declare to have no competing interests.

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**Author contributions:**

ADGM, HN and JJMM conceived the study, participated in its design, helped to draft the manuscript and mentored TBGC. CATZ, ADGM and JJMM critically

revised successive drafts of the manuscript and provided important intellectual input. CATZ and TBGC coordinated and supervised the integration of the manuscript. ES, IEJR, HN and ADGM contributed to developing the analytic plan proposed for this study. All authors read and approved the final manuscript.

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The protocol study will be a doctoral thesis of TBGC.

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# Genome-Wide Association Study of suicide attempt in a Mexican population: A study protocol

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# Genome-Wide Association Study of suicide attempt in a Mexican population: A study protocol

Thelma Beatriz González-Castro[1], Alma Delia Genis-Mendoza[2,3], Carlos Alfonso Tovilla-Zarate[4], José Jaime Martínez-Magaña[2], Isela Esther Juarez-Rojop[5], Emmanuel Sarmiento[3], Humberto Nicolini[*2,3]

[1] División Académica Multidisciplinaria de Jalpa de Méndez; Universidad Juárez Autónoma de Tabasco. Jalpa de Méndez, Tabasco, México.
[2] Instituto Nacional de Medicina Genómica (INMEGEN); Secretaria de Salud. Ciudad de México.
[3] Hospital Psiquiátrico Infantil "Dr. Juan N. Navarro". Ciudad de México
[4] División Académica Multidisciplinaria de Comalcalco; Universidad Juárez Autónoma de Tabasco. Comalcalco, Tabasco, México.
[5] División Académica Multidisciplinaria de Ciencias de la Salud; Universidad Juárez Autónoma de Tabasco. Villahermosa, Tabasco, México.
[a] These authors contributed equally to this work

TBGC: thelma.glez.castro@gmail.com; CATZ: alfonso_tovillaz@yahoo.com.mx; JJMM: jimy.10.06@gmail.com; IEJR: iselajuarezrojop@hotmail.com; ES: emmanuelsarmientoh@hotmail.com

TBGC and ADGM contributed equally to this paper

*Correspondence authors: Ph.D. Humberto Nicolini, National Institute of Genomic Medicine (INMEGEN), Psychiatric Care Services (SAP). Periférico Sur 4809, Arenal Tepepan, 14610, México City, México. Phone + 55 5350 1900. Email address: hnicolini@inmegen.gob.mx

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## Abstract

*Introduction*: Suicidality is a complex behavior and a major health problem; the specific features that could predispose to suicidal behavior have been extensively investigated, most frequently in European and Asian populations. Therefore, our aim is to present a protocol that will explore suicide attempt in Mexican individuals diagnosed with psychiatric disorders, through a genome-wide association study.

*Method and analysis*: we will perform a GWAS by comparing 700 individuals who have suicide attempt history, with control subjects without suicide attempt history (n=500). The genotyping will be conducted using the Infinium PsychArray BeadChip and quality controls will be applied to SNPs genotyped. After that, we will perform the imputation using reference panels provided by the Haplotype Reference Consortium. We will perform two different workflows: A) the classic GWAS analysis applying the same weight to all the variants and B) an algorithm with prediction of deleteriousness of variants.

*Ethics and dissemination*

This study was approved by the ethics and investigation committees of the National Institute of Genomic Medicine on July 22nd 2015, No. CEI 215/13. We plan to disseminate research findings in scientific conferences and as a manuscript in peer-reviewed journals.

*Trial registration*: CEI 215/13


Keywords: suicide & self-harm; mental health; schizophrenia & psychotic disorders; genetics.

## Article summary

Strengths and limitations of this study

- This will be the first study that aims to perform a genome wide association study to assess suicide attempt in a Mexican population.
- The methodological analysis will explore the hypothesis that there are genes that increase the risk of suicide attempt in Mexican population.
- Recruiting participants from three clinical centers in the country will give a more representative sample of the Mexican population.
- Identifying genetic variants associated with suicide attempt will improve our understanding of the biology underlying this disorder and could be useful for future microarrays designed to study Latin American populations.
- As the Mexican population has a heterogeneous genetic background, a possible limitation of this study will be that heterogeneity might interfere in the interpretation of results.

## Introduction

Suicide is one of the leading causes of death worldwide. Individuals diagnosed with psychiatric disorders have higher rates of suicide compared with the general population, which emphasizes a narrow relationship between suicide and psychiatric disorders. In this line of evidence, a considerable majority of suicide victims have had an undiagnosed psychiatric disorder at the time of death[1-3]. Furthermore, several studies consider psychiatric disorders as one of the main risk factors of suicide. Unfortunately, up to today, the mechanisms of this relationship have not been fully disentangled[4 5].

Suicidal behavior (SB), has been defined as a complex issue that results from the combination of genetic variants along with personal experiences and environmental contribution; altogether, these factors establish the disease symptomatology manifestation [1 3 6]. The results of studies based on twins, adoptions and families, support the heritability of SB, pinpointing a genetic influence[7-10]. Subsequently, many candidate-gene association studies have been performed, studying the different phenotypes of SB: suicide attempts, suicide ideation and accomplished suicide [1 3 6 11 12]. Unfortunately, SB is a polygenic trait and candidate-gene association studies underestimate the genetic background. In an attempt to search for this polygenic variation of SB, other strategies have been developed, such as the genome-wide association studies (GWAS) [6 13 14].

Over the past decade, a small number of GWAS exploring common genetic variation mostly in suicide attempters, have found significant associations between genetic components and SB, establishing possible molecular pathways involved in the susceptibility of the disease[15 16]. Although a genetic diathesis model for SB

predisposition has been proposed and GWAS have suggested candidate loci or pathways, the only information available comes from studies analyzing Caucasian or Asian populations, leaving behind Latin American populations including Mexicans[17-21]. Therefore, more studies are necessary to have a better comprehension of the SB genetic background.

Objectives

Our aim is to perform the first genome-wide association study of suicide attempters in a Mexican population, in order to explore and define the involvement of a genetic diathesis that predisposes to SB in this population. In addition, we will explore the hypothesis that there are genes and genetic variants that increase the risk of suicide attempt in Mexican population and these factors could be common in individuals with a psychiatric diagnostic.

**Methods and analysis**

*Sample population and setting*

The case group (n=700) will be formed by individuals who have had at least one suicide attempt, and this will be determined using the Structured Clinical Interview for DSM-IV (SCID-I and II) in Psychiatry out-patient areas from three clinical centers: “Dr. Gustavo A. Rovirosa” General Hospital in Tabasco, “Dr. Desiderio G. Carbajal Regional Hospital in Tabasco, Mexico and “Dr. Juan N. Navarro” Psychiatric Hospital in Mexico City. Individuals used as controls (n=500) will not have current or past history of any suicidal behavior, will not have first degree relatives with suicidal behavior history and they will be unrelated to those in the case group. Both cases

and controls will be interviewed by two psychiatrist or clinical specialists, together they will determine the presence or absence of suicide attempt. All individuals will be Mexicans with Mexican ascendancy of at least two generations (Mexican parents and grandparents); they will be recruited from several hospitals and outpatient clinics in Mexico.

*Patient and Public Involvement*

The outcomes of the present protocol will be directly communicated to patients who participate or their legal caregivers. The results will also be discussed with their psychiatrists and the corresponding health-education structures of the clinical centers. However, the patients have not directly participated in the study design or any methodological procedures.

*Ethics and dissemination*

A written informed consent will be obtained from all individuals who accept to participate. The study will be performed in accordance with the Helsinki declaration (59th General Assembly, Seoul, Korea, October 2008). This study has been already approved by the ethics and investigation committees of the National Institute of Genomic Medicine on July 22nd 2015, No. CEI 215/13. We plan to disseminate research findings in scientific conferences and as a manuscript in peer-reviewed journals.

*Clinical assessment*

Diagnoses and clinical evaluations will be performed by at least two trained senior psychiatrists of whom at least one of them would have personally examined the patient. All participants will undergo semi-structured interviews that will include life-time and family history of suicidal behavior, among other clinical features. Suicide attempt will be defined as a self-injurious act that had at least a partial intent to end one's life; the number of attempts, method used and medical damage of past suicide attempts will be gathered.

*Genotyping*

DNA will be isolated from peripheral blood leukocytes samples using a standardized protocol of the Genomic Wizard Purification Kit from Promega, as previously reported[22-24]. The integrity of genetic material will be checked on 1% agarose gels and quantified by spectrophotometry using the Nanodrop system.

The genotyping will be performed using the Infinium PsychArray BeadChip, following the manufacturer's protocol [25 26]. This array contains approximately 580000 genetic variants, wherein include a set of genetic variants previously associated with a variety of psychiatric illnesses. All genotyping analyses will be performed at the National Institute of Genomic Medicine (INMEGEN). For quality controls, we will filter-out samples and variants with call rates lower than 98% and variants deviating from Hardy-Weinberg equilibrium, with a chi-square p-value < $1x10^{-6}$. Gender concordance will be performed based on heterozygosity of X and Y chromosomes. All filtering processes will be done using the PLINK v1.9 software [27]. Then, we will perform a multidimensional scaling (MDS) analysis in PLINK, all SNPs that pass

quality controls will be pruned and used to check population stratification in order to evaluate the ancestry of the individuals included. After the MDS analysis, the first five components will be used as covariates in the association analysis. MDS dimensions will be graphically represented using the "MDS-plot" option. After quality control procedures, we will perform the imputation using reference panels provided by the Haplotype Reference Consortium r.1.1; we will use the Michigan Imputation Server for the imputation [28 29].

*Polygenic risk score calculation (PRS)*

PRS is a measurement of genetic liability to schizophrenia, based on the Psychiatric Genomics Consortium (PGC) schizophrenia GWAS[30]. SNPs will be selected and used in the PRS calculation based on p values obtained in the original PGC GWAS using PRSice[31]. PRS will be performed to search for suicide attempt associations using linear regression models adjusted by age, sex, and four multidimensional-scaling components (MDS). The estimation of gene- or set-based association tests using GWAS summary data, will be performed using Genome-wide Complex Trait Analysis (GCTA) [32 33].

*Statistical analyses*

In order to evaluate the effect of genetic variants on suicidal behavior, we will perform two workflows: A) a classic GWAS analysis applying the same importance to all the variants and B) algorithms for predicting deleteriousness of variants. Concerning the first workflow, we will conduct a mixed linear model analysis on imputed variants with a minor allele frequency of 5%. The implementation of linear

mixed models will be performed using the GCTA software [34]. For the second workflow, we will include the prediction of deliriousness of variants using different prediction algorithms, such as PolyPhen, SIFT, CADD, VEP and ENCODE [35-39]. After the functional impact on variants prediction, we will compare cases' allele frequency of loss-of-function, missense with deleterious effect, variants present in regulatory regions and variants with a PHRED-CADD score higher than 20, with the allele frequency of populations reported in the 1000 genomes and ExAC database. GWAS analysis will be performed on cases with history of suicide attempt and compared with non-suicide attempters. Genome wide significance will be set at P $<5x10^{-08}$.

*Power analysis calculation*

For the GWAS analysis, we will perform power calculations using QUANTO 1.2.4 (http://biostats.usc.edu/software). This analysis uses a log additive model of inheritance and is capable of detecting a power of 0.82 at significance threshold $5x10^{-08}$ to detect an effect size of OR ≥ 1.8 with a $P_0$ of 0.08 and MAF of 0.25, as observed in a previous study[14]. The $P_0$ that we will use is the baseline risk of suicide attempt in our population based on previous reports [40 41]. Therefore, our study will be powered to detect genetics effects.

## Discussion

Various situations and contexts have been proposed as predictors of suicidal behavior; among them, the presence of a psychiatric disorder seems to be an important determinant for such behavior. Likewise, the possible genetic

predisposition to manifest SB has been supported by several investigations, but the understanding of the precise genetic system that causes such vulnerability to suicidal tendencies is still largely incomplete. Hence, the principal aim of our protocol study is to explore the potential genetic influence on SB in a Mexican population, throughout a genome-wide association study. In addition, we want to emphasize that to our knowledge, this study protocol will be the first one to evaluate suicidal behavior in patients with schizophrenia and bipolar disorder in a Mexican population.

The majority of genetic epidemiology evidence suggest that suicidal behavior is a complex issue, where there are multiple genes that have a small effect over SB; but if combined, could become predisposing factors. Therefore, association studies that detect small effect contributions can be more useful, which is a strength of this protocol. In this sense, one of the most powerful strengths of the GWAS is that it uses many genetic markers across the whole genome to search for associations with a particular disease; as it is based on no prior assumptions, it explores a large number of genetic variants. For these reasons, we will use GWAS's results to explore the genetic influence on suicidal behavior in schizophrenics with and without SA, bipolar patients with and without SA, suicide attempters and healthy subjects as controls. Therefore, the results of this study will provide information to better comprehend the influence of the genetic background when developing suicidal behavior, among psychiatric patients.

Additionally, the findings of the present research could provide valuable information for future researchers who attempt to identify genetic risk factors of suicidal behavior, and help detect and/or treat this disease. Nowadays, there are microarrays that have been used to study several genetic variables in Caucasian and Asian populations;

to our knowledge however, there is no evidence reported of GWAS in studies that evaluate suicidal behavior in psychiatric patients. Therefore, the outcomes of the current protocol could provide essential information. The use of this type of genetic tools will allow us to identify associated SNPs, missense and insertions and indels in mental illnesses such as schizophrenia and bipolar disorder, as well as their possible participation as predictors of suicidal behavior in a Mexican population.

In conclusion, these findings could give important information to improve the design of future chips for molecular diagnosis of psychiatric disorders in Mexicans, which will be very useful in the prevention, diagnosis and prognosis of suicidal behavior in Mexico. Moreover, the findings will give a better perspective of the genetic background as a predictor of suicidal behavior in psychiatric diseases. Hence, the outcomes would be useful in genetic research as well as in prevention and early diagnosis of suicidal behavior in Mexicans

**Ethics and disseminations:**

The study will be conducted in compliance with local regulations and internationally established principles of the Declaration of Helsinki (59th General Assembly, Seoul, Korea, October 2008). This study was approved by the ethics and investigation committees of the National Institute of Genomic Medicine on July 22nd 2015, No. CEI 215/13. Before inclusion, all patients are required to sign an informed consent form.

**Consent for publication:**

Not applicable.

**Availability of data and material:**

Available upon request from the corresponding author

**Competing interest statement:**

The authors declare to have no competing interests.

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**Author contributions:**

ADGM, HN and JJMM conceived the study, participated in its design, helped to draft the manuscript and mentored TBGC. CATZ, ADGM and JJMM critically revised

successive drafts and provided important intellectual input. CATZ and TBGC coordinated and supervised the integration of the manuscript. ES, IEJR, HN and ADGM contributed to developing the analytic plan proposed for this study. All authors read and approved the final manuscript.

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