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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

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Word account: 1,850

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 death¹⁻³. 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 background⁶⁻⁹. 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

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3 the susceptibility of the disease^{15 16}. However, even if a genetic diathesis model for
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5 predisposition to SB has been proposed and GWAS have suggest candidate loci or
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7 pathways, the only information published available comes from studies analyzing
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9 Caucasians or Asians populations, leaving behind Latin American populations
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11 including Mexicans¹⁷⁻²¹. Therefore, more studies are necessary in order to have a
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13 better comprehension of the genetic background of SB.
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16 17 Objective

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20 Our aim is to present a protocol for the first genome-wide association study
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22 of suicide attempters in a Mexican population, in order to explore and define the
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24 involvement of a genetic diathesis that predispose to SB in Mexicans.
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27 28 **Methods and analysis**

29 30 *Sample population and setting*

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33 The study will include 700 Mexican cases diagnosed as schizophrenics with
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35 or without a suicide attempt and bipolar with or without a suicide attempt; also
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37 suicide attempters without psychiatric comorbidity; using Structured Clinical
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39 Interview for DSM-IV (SCID-I and II). All patients, will be recruited from three
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41 clinical centers (“Dr. Gustavo A. Rovirosa” General Hospital, “Dr. Desiderio G.
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43 Carbajal Regional Hospital in Tabasco, Mexico and “Dr. Juan N. Navarro”
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45 Psychiatric Hospital in Mexico City). A group of 500 healthy volunteers without any
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47 history of psychiatric disorders will be randomly selected as a control group. All
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49 individuals will be Mexican with Mexican ancestry at least up to two generations
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3 (Mexican grandparents); they will be recruited from several Mexican hospitals and
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5 outpatient clinics.
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8 *Ethical approval*

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11 A written informed consent will be obtained from all patients who accept to
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13 participate. The study will be performed in accordance with the Helsinki declaration
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15 (59th General Assembly, Seoul, Korea, October 2008). This study has been
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17 approved by the ethics and investigation commitments of the National Institute of
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19 Genomic Medicine on July 22nd 2015, No. CEI 215/13
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23 *Clinical assessment*

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26 The diagnoses and clinical evaluations will be performed by at least two
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28 trained senior psychiatrists of whom at least one of them would have personally
29
30 examined the patient. All the subjects included in the study will participate in semi-
31
32 structured interviews that will include life-time and family history of suicidal
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34 behavior, among other clinical features. Subsequently, the sample will be stratified
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36 as follows: schizophrenics with and without suicide attempt, bipolar with and
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38 without suicide attempt, only suicide attempters and finally, healthy subjects as
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40 controls. The suicide attempt will be defined as a self-injurious act that had at least
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42 a partial intent to end one's life; the number of attempts, method used and medical
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44 damage of past suicide attempts will be recorded.
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49 *Genotyping*

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53 The DNA will be isolated from peripheral blood leukocytes samples using a
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55 standardized protocol of the Genomic Wizard Purification Kit from Promega, as
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3 previously reported ²²⁻²⁴. The integrity of genetic material will be checked on 1%
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5 agarose gels and quantified by spectrophotometry using Nanodrop system.
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8 The genotyping will be performed using the Infinium PsychArray BeadChip,
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10 following the manufacturer's protocol ^{25 26}. This array contains approximately
11
12 580000 genetic variants, wherein include a set of genetic variants previously
13
14 associated with a variety of psychiatric illnesses. All genotyping analyses will be
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16 performed at the National Institute of Genomic Medicine (INMEGEN). For quality
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18 controls, we will filter-out samples and variants with call rates lower than 98% and
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20 variants deviating from Hardy-Weinberg equilibrium, with a chi-square p-value <
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22 1×10^{-6} . The concordance between gender will be performed based on the
23
24 heterozygosity of the X and Y chromosomes. All the filtering processes will be
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26 performed using the PLINK v1.9 software ²⁷. After the quality control procedures,
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28 we will impute variants on autosome chromosomes, with Beagle software ²⁸; during
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30 the imputation process, we will use 1000 genomes phase 3 database as
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32 references.
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39 *Statistical analyses*

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42 In order to evaluate the effect of genetic variants on suicidal behavior, we
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44 will perform two different workflows: A) a classic GWAS analysis applying the same
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46 weight to all the variants and B) an algorithm with prediction of deleteriousness of
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48 the variants. Concerning the first workflow, we will conduct a mixed linear model
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50 analysis on imputed variants with a minor allele frequency of 5%, adjusting the
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52 models with the Genetic Relationship Matrix, age and gender. The implementation
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54 of the linear mixed models will be performed on the GCTA software ²⁹. For the
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3 second workflow, will include a prediction of the deliriousness of the variants using
4 different prediction algorithms, such as PolyPhen, SIFT, CADD, VEP and
5 ENCODE³⁰⁻³⁴. After the prediction of the functional impact of the variants, we will
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7 compare the cases allele frequency of loss-of-function, missense with deleterious
8 effect, variants present in regulatory regions and variants with a PHRED-CADD
9 score higher than 20, with the allele frequency of the populations reported in the
10 1000 genomes and ExAC database.
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19 **Discussion**

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22 Various situations and contexts have been proposed as predictors of
23 suicidal behavior; among them, the presence of a psychiatric disorder seems to be
24 an important determinant for such behavior. Likewise, the possible predisposition
25 of a genetic background to manifest SB has been supported by several
26 investigations, but the understanding of the precise genetic system that causes
27 such vulnerability to suicidal tendencies is still largely incomplete. Hence, the
28 principal aim of our protocol study is to explore the potential genetic influence over
29 SB in a Mexican population, throughout a genome-wide association study. In
30 addition, we want to emphasize that to our knowledge, this study protocol will be
31 the first one to evaluate suicidal behavior in schizophrenic and bipolar disorder
32 patients, in a Mexican population.
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49 The majority of the genetic epidemiology evidence suggest that suicidal
50 behavior is a complex issue, where there are multiple genes that have a small
51 effect over SB, but if combined, could become predisposing factors; therefore,
52 association studies that detect small effect contributions can be more useful, which
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3 is a strength of this protocol. In this sense, one of the most powerful strengths of
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5 the GWAS is that uses many genetic markers across the whole genome to analyze
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7 for associations with a particular disease; as it is based on no prior assumptions, it
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9 explores a large number of genetic variants. For these reasons, we will use a
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11 GWAS to explore the genetic influence over suicidal behavior in schizophrenics
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13 with and without SA, bipolar patients with and without SA, suicide attempters and
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15 healthy subjects as controls. Therefore, the results of this study will provide
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17 information to better comprehend the influence of the genetic background in the
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19 development of suicidal behavior, among psychiatric patients.
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24 Additionally, the findings of the present research could provide valuable information
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26 for future researches that attempt to identify genetic risk factors for suicidal
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28 behavior, and help detect and/or treat this disease. Nowadays, there are
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30 microarrays that have been used to study several genetic variables in Caucasians
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32 and Asians; but to our knowledge, there is no evidence reported of GWAS in
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34 studies that evaluate suicidal behavior in psychiatric patients. Therefore, the
35
36 outcomes of the study derived of the current protocol, could provide essential
37
38 information, because the use of this type of genetic tools will allow us to identify the
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40 associated SNPs, missense and insertions and indels in mental illnesses such as
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42 schizophrenia and bipolar disorder and their possible participation as predictors of
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44 suicidal behavior in a Mexican population. Consequently, these findings could give
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46 important information to improve the design of future chips for molecular diagnosis
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48 of psychiatric disorders in Mexicans, which will be very useful in the prevention,
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50 diagnosis and prognosis of suicidal behavior in Mexico.
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3 In conclusion, the completion of the present protocol could impulse the
4 design of a microarray that includes associated variants to SB in Mexican
5 population. Moreover, the findings will give a better perspective of the participation
6 of the genetic background as a predictor of suicidal behavior in psychiatric
7 diseases. Hence, the outcomes would be useful in genetic research, as well as in
8 prevention and early diagnosis of suicidal behavior in Mexicans.
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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 statement:

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

1
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3 and TBGC coordinated and supervised the integration of the manuscript. All
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5 authors read and approved the final manuscript.
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7

8 **Acknowledgements** 9

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11 The protocol study will be a doctoral thesis of TBGC.
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References

1. Perlis RH, Huang J, Purcell S, et al. Genome-wide association study of suicide attempts in mood disorder patients. *The American journal of psychiatry* 2010;167(12):1499-507. doi: 10.1176/appi.ajp.2010.10040541 [published Online First: 2010/11/03]
2. Pulay AJ, Rethelyi JM. Multimarker analysis suggests the involvement of BDNF signaling and microRNA biosynthesis in suicidal behavior. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics* 2016;171(6):763-76. doi: 10.1002/ajmg.b.32433 [published Online First: 2016/02/28]
3. Mullins N, Perroud N, Uher R, et al. Genetic relationships between suicide attempts, suicidal ideation and major psychiatric disorders: a genome-wide association and polygenic scoring study. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics* 2014;165B(5):428-37. doi: 10.1002/ajmg.b.32247 [published Online First: 2014/06/26]
4. Perroud N, Uher R, Ng MY, et al. Genome-wide association study of increasing suicidal ideation during antidepressant treatment in the GENDEP project. *Pharmacogenomics J* 2012;12(1):68-77. doi: 10.1038/tpj.2010.70 [published Online First: 2010/09/30]
5. Stein MB, Ware EB, Mitchell C, et al. Genomewide association studies of suicide attempts in US soldiers. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics* 2017;174(8):786-97. doi: 10.1002/ajmg.b.32594 [published Online First: 2017/09/14]
6. Levine SZ, Goldberg Y, Yoffe R, et al. Suicide attempts in a national population of twins concordant for psychoses. *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology* 2014;24(8):1203-9. doi: 10.1016/j.euroneuro.2014.05.014 [published Online First: 2014/06/28]
7. Linker J, Gillespie NA, Maes H, et al. Suicidal ideation, depression, and conduct disorder in a sample of adolescent and young adult twins. *Suicide & life-threatening behavior* 2012;42(4):426-36. doi: 10.1111/j.1943-278X.2012.00101.x [published Online First: 2012/06/01]
8. Petersen L, Sorensen TI, Kragh Andersen P, et al. Genetic and familial environmental effects on suicide attempts: a study of Danish adoptees and their biological and adoptive siblings. *Journal of affective disorders* 2014;155:273-7. doi: 10.1016/j.jad.2013.11.012 [published Online First: 2013/12/05]
9. Roy A, Rylander G, Sarchiapone M. Genetics of suicides. Family studies and molecular genetics. *Annals of the New York Academy of Sciences* 1997;836:135-57. [published Online First: 1998/06/09]
10. Mirkovic B, Cohen D, Laurent C, et al. A case-control association study of 12 candidate genes and attempted suicide in French adolescents. *International journal of adolescent medicine and health* 2017 doi: 10.1515/ijamh-2017-0089 [published Online First: 2017/09/14]
11. Tombacz D, Maroti Z, Kalmar T, et al. High-Coverage Whole-Exome Sequencing Identifies Candidate Genes for Suicide in Victims with Major Depressive Disorder. *Scientific reports* 2017;7(1):7106. doi: 10.1038/s41598-017-06522-3 [published Online First: 2017/08/05]
12. Sokolowski M, Wasserman J, Wasserman D. Rare CNVs in Suicide Attempt include Schizophrenia-Associated Loci and Neurodevelopmental Genes: A Pilot Genome-Wide and Family-Based Study. *PLoS one* 2016;11(12):e0168531. doi: 10.1371/journal.pone.0168531 [published Online First: 2016/12/29]

13. Gross JA, Bureau A, Croteau J, et al. A genome-wide copy number variant study of suicidal behavior. *PloS one* 2015;10(5):e0128369. doi: 10.1371/journal.pone.0128369 [published Online First: 2015/05/27]
14. Galfalvy H, Haghighi F, Hodgkinson C, et al. A genome-wide association study of suicidal behavior. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics* 2015;168(7):557-63. doi: 10.1002/ajmg.b.32330 [published Online First: 2015/06/17]
15. Galfalvy H, Zalsman G, Huang YY, et al. A pilot genome wide association and gene expression array study of suicide with and without major depression. *The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry* 2013;14(8):574-82. doi: 10.3109/15622975.2011.597875 [published Online First: 2011/11/09]
16. Laje G, Allen AS, Akula N, et al. Genome-wide association study of suicidal ideation emerging during citalopram treatment of depressed outpatients. *Pharmacogenet Genomics* 2009;19(9):666-74. doi: 10.1097/FPC.0b013e32832e4bcd [published Online First: 2009/09/03]
17. Zai CC, Goncalves VF, Tiwari AK, et al. A genome-wide association study of suicide severity scores in bipolar disorder. *Journal of psychiatric research* 2015;65:23-9. doi: 10.1016/j.jpsychires.2014.11.002 [published Online First: 2015/04/29]
18. Menke A, Domschke K, Czamara D, et al. Genome-wide association study of antidepressant treatment-emergent suicidal ideation. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 2012;37(3):797-807. doi: 10.1038/npp.2011.257 [published Online First: 2011/10/28]
19. Bani-Fatemi A, Graff A, Zai C, et al. GWAS analysis of suicide attempt in schizophrenia: Main genetic effect and interaction with early life trauma. *Neurosci Lett* 2016;622:102-6. doi: 10.1016/j.neulet.2016.04.043 [published Online First: 2016/04/26]
20. Schosser A, Butler AW, Ising M, et al. Genomewide association scan of suicidal thoughts and behaviour in major depression. *PloS one* 2011;6(7):e20690. doi: 10.1371/journal.pone.0020690 [published Online First: 2011/07/14]
21. Willour VL, Seifuddin F, Mahon PB, et al. A genome-wide association study of attempted suicide. *Molecular psychiatry* 2012;17(4):433-44. doi: 10.1038/mp.2011.4 [published Online First: 2011/03/23]
22. Gonzalez-Castro TB, Nicolini H, Lanzagorta N, et al. The role of brain-derived neurotrophic factor (BDNF) Val66Met genetic polymorphism in bipolar disorder: a case-control study, comorbidities, and meta-analysis of 16,786 subjects. *Bipolar disorders* 2015;17(1):27-38. doi: 10.1111/bdi.12227 [published Online First: 2014/07/22]
23. Gonzalez-Castro TB, Tovilla-Zarate C, Juarez-Rojop I, et al. Association of the 5HTR2A gene with suicidal behavior: case-control study and updated meta-analysis. *BMC psychiatry* 2013;13:25. doi: 10.1186/1471-244x-13-25 [published Online First: 2013/01/15]
24. Gonzalez-Castro TB, Tovilla-Zarate CA, Juarez-Rojop I, et al. Association of 5HTR1A gene variants with suicidal behavior: case-control study and updated meta-analysis. *Journal of psychiatric research* 2013;47(11):1665-72. doi: 10.1016/j.jpsychires.2013.04.011 [published Online First: 2013/08/06]
25. Guo Y, He J, Zhao S, et al. Illumina human exome genotyping array clustering and quality control. *Nature protocols* 2014;9(11):2643-62. doi: 10.1038/nprot.2014.174 [published Online First: 2014/10/17]
26. Borges G, Orozco R, Medina Mora ME. [Risk index for attempted suicide in Mexico]. *Salud publica de Mexico* 2012;54(6):595-606. [published Online First: 2013/01/16]

- 1
2
3 27. Chang CC, Chow CC, Tellier LC, et al. Second-generation PLINK: rising to the challenge of larger
4 and richer datasets. *GigaScience* 2015;4:7. doi: 10.1186/s13742-015-0047-8 [published
5 Online First: 2015/02/28]
6
7 28. Browning BL, Browning SR. Genotype Imputation with Millions of Reference Samples. *Am J*
8 *Hum Genet* 2016;98(1):116-26. doi: 10.1016/j.ajhg.2015.11.020 [published Online First:
9 2016/01/11]
10
11 29. Yang J, Lee SH, Goddard ME, et al. Genome-wide complex trait analysis (GCTA): methods, data
12 analyses, and interpretations. *Methods in molecular biology (Clifton, NJ)* 2013;1019:215-
13 36. doi: 10.1007/978-1-62703-447-0_9 [published Online First: 2013/06/13]
14
15 30. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations
16 using PolyPhen-2. *Current protocols in human genetics* 2013;Chapter 7:Unit7.20. doi:
17 10.1002/0471142905.hg0720s76 [published Online First: 2013/01/15]
18
19 31. Ng PC, Henikoff S. Predicting deleterious amino acid substitutions. *Genome research*
20 2001;11(5):863-74. doi: 10.1101/gr.176601 [published Online First: 2001/05/05]
21
22 32. McLaren W, Gil L, Hunt SE, et al. The Ensembl Variant Effect Predictor. *Genome biology*
23 2016;17(1):122. doi: 10.1186/s13059-016-0974-4 [published Online First: 2016/06/09]
24
25 33. An integrated encyclopedia of DNA elements in the human genome. *Nature*
26 2012;489(7414):57-74. doi: 10.1038/nature11247 [published Online First: 2012/09/08]
27
28 34. Kircher M, Witten DM, Jain P, et al. A general framework for estimating the relative
29 pathogenicity of human genetic variants. 2014;46(3):310-5. doi: 10.1038/ng.2892
30
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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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2	Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted
3			estimates and their precision (eg, 95% confidence interval). Make clear
4			which confounders were adjusted for and why they were included
5			(b) Report category boundaries when continuous variables were categorized
6			(c) If relevant, consider translating estimates of relative risk into absolute risk
7			for a meaningful time period
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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>.

BMJ Open

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

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SCHOLARONE™
Manuscripts

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3 **Genome-Wide Association Study of suicide attempt in a Mexican population:**
4 **A study protocol**
5

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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.

1 Introduction

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

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

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

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3 25 predisposition to SB has been proposed and GWAS have suggested candidate loci
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5 26 or pathways, the only information available comes from studies analyzing
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7 27 Caucasians or Asians populations, leaving behind Latin American populations
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9 28 including Mexicans¹⁷⁻²¹. Therefore, more studies are necessary in order to have a
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11 29 better comprehension of the genetic background of SB.
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16 17 31 Objective

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19 32 Our aim is to perform the first genome-wide association study of suicide
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21 33 attempters in a Mexican population, in order to explore and define the involvement
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23 34 of a genetic diathesis that predisposes to SB in this population. In addition, we will
24
25 35 explore the hypothesis that there are genes and genetic variants that increase the
26
27 36 risk of suicide attempt in Mexican population and these factors could be common in
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29 37 individuals with a psychiatric diagnostic.
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35 39 **Methods and analysis**

36 37 40 *Sample population and setting*

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40 41 The case group (n=700) will be formed by individuals who have had at least one
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42 42 suicide attempt, and this will be determined using Structured Clinical Interview for
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44 43 DSM-IV (SCID-I and II) in the Psychiatry out-patient area from three clinical
45
46 44 centers: “Dr. Gustavo A. Rovirosa” General Hospital in Tabasco, “Dr. Desiderio G.
47
48 45 Carbajal Regional Hospital in Tabasco, Mexico and “Dr. Juan N. Navarro”
49
50 46 Psychiatric Hospital in Mexico City. Individuals used as controls (n=500) will not
51
52 47 have current or past history of any suicidal behavior, with no first degree relatives
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54 48 with a history of suicidal behavior and they will have to be unrelated to those in the
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3 49 case group. Both cases and controls will be interviewed by two psychiatrist or
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5 50 clinical specialists, together will determine the presence or absence of suicide
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7 51 attempt. All individuals will be Mexican with Mexican ascendancy of at least two
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9 52 generations (Mexican parents and grandparents); they will be recruited from
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11 53 several Mexican hospitals and outpatient clinics.
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17 54 18 *Patient and Public Involvement*

19 56 The outcomes of the present protocol will be directly communicated to the patients
20
21 57 who participate or their legal caregivers. The results will also be discussed with
22
23 58 their psychiatrists and the corresponding health-education structures of the clinical
24
25 59 centers. However, the patients have not been directly participate in the study
26
27 60 design or any methodological procedures. Nevertheless, the authors of this
28
29 61 protocol have clinical experience with SB patients and their families, which helped
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31 62 designed this study.
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37 63 38 *Ethics and dissemination*

39 64
40 65 A written informed consent will be obtained from all individuals who accept
41
42 66 to participate. The study will be performed in accordance with the Helsinki
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44 67 declaration (59th General Assembly, Seoul, Korea, October 2008). This study was
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46 68 approved by the ethics and investigation commitments of the National Institute of
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48 69 Genomic Medicine on July 22nd 2015, No. CEI 215/13. We plan to disseminate
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50 70 research findings through scientific conferences and as a manuscript in peer-
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52 71 reviewed journals.
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3 73 *Clinical assessment*
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5 74 Diagnoses and clinical evaluations will be performed by at least two trained
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7
8 75 senior psychiatrists of whom at least one of them would have personally examined
9
10 76 the patient. All participants will undergo semi-structured interviews that will include
11
12 77 life-time and family history of suicidal behavior, among other clinical features.
13
14 78 Suicide attempt will be defined as a self-injurious act that had at least a partial
15
16 79 intent to end one's life; the number of attempts, method used and medical damage
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18
19 80 of past suicide attempts will be obtained.
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24 82 *Genotyping*
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26 83 The DNA will be isolated from peripheral blood leukocytes samples using a
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28 84 standardized protocol of the Genomic Wizard Purification Kit from Promega, as
29
30 85 previously reported ²²⁻²⁴. The integrity of genetic material will be checked on 1%
31
32 86 agarose gels and quantified by spectrophotometry using Nanodrop system.
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35 87 The genotyping will be performed using the Infinium PsychArray BeadChip,
36
37 88 following the manufacturer's protocol ^{25 26}. This array contains approximately
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39 89 580000 genetic variants, wherein include a set of genetic variants previously
40
41 90 associated with a variety of psychiatric illnesses. All genotyping analyses will be
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43 91 performed at the National Institute of Genomic Medicine (INMEGEN). For quality
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45 92 controls, we will filter-out samples and variants with call rates lower than 98% and
46
47 93 variants deviating from Hardy-Weinberg equilibrium, with a chi-square p-value <
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49 94 1×10^{-6} . The concordance between gender will be performed based on the
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51 95 heterozygosity of the X and Y chromosomes. All the filtering processes will be
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53 96 performed using the PLINK v1.9 software ²⁷. After that, we will perform a
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3 97 multidimensional scaling (MDS) analysis in PLINK, all SNPs that pass quality
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5 98 controls will be pruned and used to check population stratification in order to
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7 99 evaluate the ancestry of individuals included. After the MDS analysis, the first five
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10 100 components will be used as covariates in the association analysis. MDS
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12 101 dimensions will be graphically represented using the “MDS-plot” option. After
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14 102 quality control procedures, we will perform the imputation using the reference
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16 103 panels provided by the Haplotype Reference Consortium r.1.1; we will use the
17
18 104 Michigan Imputation Server for the imputation^{28 29}.

105

106 *Polygenic risk score calculation (PRS)*

107 PRS is a measurement of genetic liability to schizophrenia based on the
108 Psychiatric Genomics Consortium (PGC) schizophrenia GWAS³⁰. SNPs will be
109 selected to be included in the PRS calculation based on p values obtained in the
110 original PGC GWAS using PRSice³¹. PRS will be used to test an association with
111 suicide attempt using linear regression models adjusted by age, sex, and four
112 multidimensional-scaling components (MDS). The estimation of gene- or set-based
113 association tests using GWAS summary data, will be performed using Genome-
114 wide Complex Trait Analysis (GCTA)^{32 33}.

115

116 *Statistical analyses*

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

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3 121 mixed linear model analysis on imputed variants with a minor allele frequency of
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5 122 5%. The implementation of linear mixed models will be performed on the GCTA
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7 123 software³⁴. For the second workflow, will include a prediction of the deliriousness
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10 124 of variants using different prediction algorithms, such as PolyPhen, SIFT, CADD,
11
12 125 VEP and ENCODE³⁵⁻³⁹. After the prediction of the functional impact of the
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14 126 variants, we will compare the cases allele frequency of loss-of-function, missense
15
16 127 with deleterious effect, variants present in regulatory regions and variants with a
17
18 128 PHRED-CADD score higher than 20, with the allele frequency of the populations
19
20 129 reported in the 1000 genomes and ExAC database. GWAS analysis will be
21
22 130 performed on cases with a history of suicide attempts and compared to non-suicide
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24 131 attempters. For the joint analysis, we will investigate the possibility of a new
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26 132 association within the genome; therefore, multiple testing corrections will be
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28 133 conducted using the Bonferroni correction and permutation; the corrected p value
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30 134 will depend on the total number of independent tests.
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136 *Power analysis calculation*

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39 137 For the primary analysis of the GWAS suicide attempt using 700 cases and
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41 138 500 controls, we will use a log additive model of inheritance, MAF of 25%, P_0 of
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43 139 0.08 the power will be 0.99. All the calculations will be performed using QUANTO
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45 140 1.2.4. (<http://biostats.usc.edu/software>), as previously reported¹⁴.
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51 **Discussion**

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53 143 Various situations and contexts have been proposed as predictors of
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55 144 suicidal behavior; among them, the presence of a psychiatric disorder seems to be
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3 145 an important determinant for such behavior. Likewise, the possible predisposition
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5 146 of a genetic background to manifest SB has been supported by several
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7 147 investigations, but the understanding of the precise genetic system that causes
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9 148 such vulnerability to suicidal tendencies is still largely incomplete. Hence, the
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11 149 principal aim of our protocol study is to explore the potential genetic influence on
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13 150 SB in a Mexican population, throughout a genome-wide association study. In
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15 151 addition, we want to emphasize that to our knowledge, this study protocol will be
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17 152 the first one to evaluate suicidal behavior in patients with schizophrenia and bipolar
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19 153 disorder in a Mexican population.
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23
24 154 The majority of the genetic epidemiology evidence suggest that suicidal
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26 155 behavior is a complex issue, where there are multiple genes that have a small
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28 156 effect over SB; but if combined, could become predisposing factors. Therefore,
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30 157 association studies that detect small effect contributions can be more useful, which
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32 158 is a strength of this protocol. In this sense, one of the most powerful strengths of
33
34 159 the GWAS is that it uses many genetic markers across the whole genome to
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36 160 search for associations with a particular disease; as it is based on no prior
37
38 161 assumptions, it explores a large number of genetic variants. For these reasons, we
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40 162 will use GWAS to explore the genetic influence on suicidal behavior in
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42 163 schizophrenics with and without SA, bipolar patients with and without SA, suicide
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44 164 attempters and healthy subjects as controls. Therefore, the results of this study will
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46 165 provide information to better comprehend the influence of the genetic background
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48 166 on the development of suicidal behavior, among psychiatric patients.
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53 167 Additionally, the findings of the present research could provide valuable information
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55 168 for future researches who attempt to identify genetic risk factors of suicidal
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3 169 behavior, and help detect and/or treat this disease. Nowadays, there are
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5 170 microarrays that have been used to study several genetic variables in Caucasian
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7 171 and Asian populations; but to our knowledge, there is no evidence reported of
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9 172 GWAS in studies that evaluate suicidal behavior in psychiatric patients. Therefore,
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11 173 the outcomes of the study derived of the current protocol, could provide essential
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13 174 information. The use of this type of genetic tools will allow us to identify associated
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15 175 SNPs, missense and insertions and indels in mental illnesses such as
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17 176 schizophrenia and bipolar disorder, as well as their possible participation as
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19 177 predictors of suicidal behavior in a Mexican population. Consequently, these
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21 178 findings could give important information to improve the design of future chips for
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23 179 molecular diagnosis of psychiatric disorders in Mexicans, which will be very useful
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25 180 in the prevention, diagnosis and prognosis of suicidal behavior in Mexico.
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31 181 In conclusion, the completion of the present protocol could impulse the
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33 182 design of a microarray that includes associated variants to SB in Mexican
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35 183 population. Moreover, the findings will give a better perspective of the participation
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37 184 of the genetic background as a predictor of suicidal behavior in psychiatric
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39 185 diseases. Hence, the outcomes would be useful in genetic research, as well as in
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41 186 prevention and early diagnosis of suicidal behavior in Mexicans
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3 188 **Ethics and disseminations:**
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5 189 The study will be conducted in compliance with local regulations and internationally
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7 190 established principles of the Declaration of Helsinki (59th General Assembly,
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9 191 Seoul, Korea, October 2008). This study was approved by the ethics and
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11 192 investigation commitments of the National Institute of Genomic Medicine on July
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13 193 22nd 2015, No. CEI 215/13. Before inclusion, all patients are required to sign an
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15 194 informed consent form.
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19 195 **Consent for publication:**
20

21 196 Not applicable.
22

23
24 197 **Availability of data and material:**
25

26 198 Not applicable.
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28
29 199 **Competing interest statement:**
30

31 200 The authors declare to have no competing interests.
32

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34

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37 203 Humberto Nicolini.
38

39
40 204 **Author contributions:**
41

42 205 AGM, HN and JJMM conceived the study, participated in its design, helped to draft
43
44 206 the manuscript and mentored TBGC. CATZ, AGM and JJMM critically revised
45
46 207 successive drafts of the manuscript and provided important intellectual input. CATZ
47
48 208 and TBGC coordinated and supervised the integration of the manuscript. All
49
50 209 authors read and approved the final manuscript.
51
52

53
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55

56 211 The protocol study will be a doctoral thesis of TBGC.
57
58
59
60

212 **References**

- 213 1. Perlis RH, Huang J, Purcell S, et al. Genome-wide association study of suicide attempts in mood
214 disorder patients. *The American journal of psychiatry* 2010;167(12):1499-507. doi:
215 10.1176/appi.ajp.2010.10040541 [published Online First: 2010/11/03]
- 216 2. Pulay AJ, Rethelyi JM. Multimarker analysis suggests the involvement of BDNF signaling and
217 microRNA biosynthesis in suicidal behavior. *American journal of medical genetics Part B,*
218 *Neuropsychiatric genetics : the official publication of the International Society of*
219 *Psychiatric Genetics* 2016;171(6):763-76. doi: 10.1002/ajmg.b.32433 [published Online
220 First: 2016/02/28]
- 221 3. Mullins N, Perroud N, Uher R, et al. Genetic relationships between suicide attempts, suicidal
222 ideation and major psychiatric disorders: a genome-wide association and polygenic scoring
223 study. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official*
224 *publication of the International Society of Psychiatric Genetics* 2014;165B(5):428-37. doi:
225 10.1002/ajmg.b.32247 [published Online First: 2014/06/26]
- 226 4. Perroud N, Uher R, Ng MY, et al. Genome-wide association study of increasing suicidal ideation
227 during antidepressant treatment in the GENDEP project. *Pharmacogenomics J*
228 2012;12(1):68-77. doi: 10.1038/tpj.2010.70 [published Online First: 2010/09/30]
- 229 5. Stein MB, Ware EB, Mitchell C, et al. Genomewide association studies of suicide attempts in US
230 soldiers. *American journal of medical genetics Part B, Neuropsychiatric genetics : the*
231 *official publication of the International Society of Psychiatric Genetics* 2017;174(8):786-97.
232 doi: 10.1002/ajmg.b.32594 [published Online First: 2017/09/14]
- 233 6. Levine SZ, Goldberg Y, Yoffe R, et al. Suicide attempts in a national population of twins
234 concordant for psychoses. *European neuropsychopharmacology : the journal of the*
235 *European College of Neuropsychopharmacology* 2014;24(8):1203-9. doi:
236 10.1016/j.euroneuro.2014.05.014 [published Online First: 2014/06/28]
- 237 7. Linker J, Gillespie NA, Maes H, et al. Suicidal ideation, depression, and conduct disorder in a
238 sample of adolescent and young adult twins. *Suicide & life-threatening behavior*
239 2012;42(4):426-36. doi: 10.1111/j.1943-278X.2012.00101.x [published Online First:
240 2012/06/01]
- 241 8. Petersen L, Sorensen TI, Kragh Andersen P, et al. Genetic and familial environmental effects on
242 suicide attempts: a study of Danish adoptees and their biological and adoptive siblings.
243 *Journal of affective disorders* 2014;155:273-7. doi: 10.1016/j.jad.2013.11.012 [published
244 Online First: 2013/12/05]
- 245 9. Roy A, Rylander G, Sarchiapone M. Genetics of suicides. Family studies and molecular genetics.
246 *Annals of the New York Academy of Sciences* 1997;836:135-57. [published Online First:
247 1998/06/09]
- 248 10. Mirkovic B, Cohen D, Laurent C, et al. A case-control association study of 12 candidate genes
249 and attempted suicide in French adolescents. *International journal of adolescent medicine*
250 *and health* 2017 doi: 10.1515/ijamh-2017-0089 [published Online First: 2017/09/14]
- 251 11. Tombacz D, Maroti Z, Kalmar T, et al. High-Coverage Whole-Exome Sequencing Identifies
252 Candidate Genes for Suicide in Victims with Major Depressive Disorder. *Scientific reports*
253 2017;7(1):7106. doi: 10.1038/s41598-017-06522-3 [published Online First: 2017/08/05]
- 254 12. Sokolowski M, Wasserman J, Wasserman D. Rare CNVs in Suicide Attempt include
255 Schizophrenia-Associated Loci and Neurodevelopmental Genes: A Pilot Genome-Wide and
256 Family-Based Study. *PloS one* 2016;11(12):e0168531. doi: 10.1371/journal.pone.0168531
257 [published Online First: 2016/12/29]

- 1
2
3 258 13. Gross JA, Bureau A, Croteau J, et al. A genome-wide copy number variant study of suicidal
4 259 behavior. *PloS one* 2015;10(5):e0128369. doi: 10.1371/journal.pone.0128369 [published
5 260 Online First: 2015/05/27]
6 261 14. Galfalvy H, Haghghi F, Hodgkinson C, et al. A genome-wide association study of suicidal
7 262 behavior. *American journal of medical genetics Part B, Neuropsychiatric genetics : the*
8 263 *official publication of the International Society of Psychiatric Genetics* 2015;168(7):557-63.
9 264 doi: 10.1002/ajmg.b.32330 [published Online First: 2015/06/17]
10 265 15. Galfalvy H, Zalsman G, Huang YY, et al. A pilot genome wide association and gene expression
11 266 array study of suicide with and without major depression. *The world journal of biological*
12 267 *psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry*
13 268 2013;14(8):574-82. doi: 10.3109/15622975.2011.597875 [published Online First:
14 269 2011/11/09]
15 270 16. Laje G, Allen AS, Akula N, et al. Genome-wide association study of suicidal ideation emerging
16 271 during citalopram treatment of depressed outpatients. *Pharmacogenet Genomics*
17 272 2009;19(9):666-74. doi: 10.1097/FPC.0b013e32832e4bcd [published Online First:
18 273 2009/09/03]
19 274 17. Zai CC, Goncalves VF, Tiwari AK, et al. A genome-wide association study of suicide severity
20 275 scores in bipolar disorder. *Journal of psychiatric research* 2015;65:23-9. doi:
21 276 10.1016/j.jpsychires.2014.11.002 [published Online First: 2015/04/29]
22 277 18. Menke A, Domschke K, Czamara D, et al. Genome-wide association study of antidepressant
23 278 treatment-emergent suicidal ideation. *Neuropsychopharmacology : official publication of*
24 279 *the American College of Neuropsychopharmacology* 2012;37(3):797-807. doi:
25 280 10.1038/npp.2011.257 [published Online First: 2011/10/28]
26 281 19. Bani-Fatemi A, Graff A, Zai C, et al. GWAS analysis of suicide attempt in schizophrenia: Main
27 282 genetic effect and interaction with early life trauma. *Neurosci Lett* 2016;622:102-6. doi:
28 283 10.1016/j.neulet.2016.04.043 [published Online First: 2016/04/26]
29 284 20. Schosser A, Butler AW, Ising M, et al. Genomewide association scan of suicidal thoughts and
30 285 behaviour in major depression. *PloS one* 2011;6(7):e20690. doi:
31 286 10.1371/journal.pone.0020690 [published Online First: 2011/07/14]
32 287 21. Willour VL, Seifuddin F, Mahon PB, et al. A genome-wide association study of attempted
33 288 suicide. *Molecular psychiatry* 2012;17(4):433-44. doi: 10.1038/mp.2011.4 [published
34 289 Online First: 2011/03/23]
35 290 22. Gonzalez-Castro TB, Nicolini H, Lanzagorta N, et al. The role of brain-derived neurotrophic
36 291 factor (BDNF) Val66Met genetic polymorphism in bipolar disorder: a case-control study,
37 292 comorbidities, and meta-analysis of 16,786 subjects. *Bipolar disorders* 2015;17(1):27-38.
38 293 doi: 10.1111/bdi.12227 [published Online First: 2014/07/22]
39 294 23. Gonzalez-Castro TB, Tovilla-Zarate C, Juarez-Rojop I, et al. Association of the 5HTR2A gene with
40 295 suicidal behavior: case-control study and updated meta-analysis. *BMC psychiatry*
41 296 2013;13:25. doi: 10.1186/1471-244x-13-25 [published Online First: 2013/01/15]
42 297 24. Gonzalez-Castro TB, Tovilla-Zarate CA, Juarez-Rojop I, et al. Association of 5HTR1A gene
43 298 variants with suicidal behavior: case-control study and updated meta-analysis. *Journal of*
44 299 *psychiatric research* 2013;47(11):1665-72. doi: 10.1016/j.jpsychires.2013.04.011
45 300 [published Online First: 2013/08/06]
46 301 25. Guo Y, He J, Zhao S, et al. Illumina human exome genotyping array clustering and quality
47 302 control. *Nature protocols* 2014;9(11):2643-62. doi: 10.1038/nprot.2014.174 [published
48 303 Online First: 2014/10/17]
49 304 26. Borges G, Orozco R, Medina Mora ME. [Risk index for attempted suicide in Mexico]. *Salud*
50 305 *publica de Mexico* 2012;54(6):595-606. [published Online First: 2013/01/16]
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3 306 27. Chang CC, Chow CC, Tellier LC, et al. Second-generation PLINK: rising to the challenge of larger
4 307 and richer datasets. *GigaScience* 2015;4:7. doi: 10.1186/s13742-015-0047-8 [published
5 308 Online First: 2015/02/28]
6 309 28. Loh PR, Danecek P, Palamara PF, et al. Reference-based phasing using the Haplotype Reference
7 310 Consortium panel. *Nat Genet* 2016;48(11):1443-48. doi: 10.1038/ng.3679 [published
8 311 Online First: 2016/10/28]
9 312 29. McCarthy S, Das S, Kretzschmar W, et al. A reference panel of 64,976 haplotypes for genotype
10 313 imputation. *Nat Genet* 2016;48(10):1279-83. doi: 10.1038/ng.3643 [published Online First:
11 314 2016/08/23]
12 315 30. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 2014;511(7510):421-
13 316 7. doi: 10.1038/nature13595 [published Online First: 2014/07/25]
14 317 31. Euesden J, Lewis CM, O'Reilly PF. PRSice: Polygenic Risk Score software. *Bioinformatics*
15 318 2015;31(9):1466-8. doi: 10.1093/bioinformatics/btu848 [published Online First:
16 319 2015/01/01]
17 320 32. Yang J, Lee SH, Goddard ME, et al. GCTA: a tool for genome-wide complex trait analysis. *Am J*
18 321 *Hum Genet* 2011;88(1):76-82. doi: 10.1016/j.ajhg.2010.11.011 [published Online First:
19 322 2010/12/21]
20 323 33. Bakshi A, Zhu Z, Vinkhuyzen AA, et al. Fast set-based association analysis using summary data
21 324 from GWAS identifies novel gene loci for human complex traits. *Sci Rep* 2016;6:32894. doi:
22 325 10.1038/srep32894 [published Online First: 2016/09/09]
23 326 34. Yang J, Lee SH, Goddard ME, et al. Genome-wide complex trait analysis (GCTA): methods, data
24 327 analyses, and interpretations. *Methods in molecular biology (Clifton, NJ)* 2013;1019:215-
25 328 36. doi: 10.1007/978-1-62703-447-0_9 [published Online First: 2013/06/13]
26 329 35. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations
27 330 using PolyPhen-2. *Current protocols in human genetics* 2013;Chapter 7:Unit7.20. doi:
28 331 10.1002/0471142905.hg0720s76 [published Online First: 2013/01/15]
29 332 36. Ng PC, Henikoff S. Predicting deleterious amino acid substitutions. *Genome research*
30 333 2001;11(5):863-74. doi: 10.1101/gr.176601 [published Online First: 2001/05/05]
31 334 37. McLaren W, Gil L, Hunt SE, et al. The Ensembl Variant Effect Predictor. *Genome biology*
32 335 2016;17(1):122. doi: 10.1186/s13059-016-0974-4 [published Online First: 2016/06/09]
33 336 38. An integrated encyclopedia of DNA elements in the human genome. *Nature*
34 337 2012;489(7414):57-74. doi: 10.1038/nature11247 [published Online First: 2012/09/08]
35 338 39. Kircher M, Witten DM, Jain P, et al. A general framework for estimating the relative
36 339 pathogenicity of human genetic variants. 2014;46(3):310-5. doi: 10.1038/ng.2892
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BMJ Open

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

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Manuscripts

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3 **Genome-Wide Association Study of suicide attempt in a Mexican population:**
4 **A study protocol**
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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.

1 Introduction

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

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

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

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3 25 predisposition to SB has been proposed and GWAS have suggested candidate loci
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5 26 or pathways, the only information available comes from studies analyzing
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7 27 Caucasians or Asians populations, leaving behind Latin American populations
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9 28 including Mexicans¹⁷⁻²¹. Therefore, more studies are necessary in order to have a
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11 29 better comprehension of the genetic background of SB.
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16 17 31 Objective

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19 32 Our aim is to perform the first genome-wide association study of suicide
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21 33 attempters in a Mexican population, in order to explore and define the involvement
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23 34 of a genetic diathesis that predisposes to SB in this population. In addition, we will
24
25 35 explore the hypothesis that there are genes and genetic variants that increase the
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27 36 risk of suicide attempt in Mexican population and these factors could be common in
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29 37 individuals with a psychiatric diagnostic.
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35 39 **Methods and analysis**

36 37 40 *Sample population and setting*

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40 41 The case group (n=700) will be formed by individuals who have had at least one
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42 42 suicide attempt, and this will be determined using Structured Clinical Interview for
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44 43 DSM-IV (SCID-I and II) in the Psychiatry out-patient area from three clinical
45
46 44 centers: “Dr. Gustavo A. Roviroso” General Hospital in Tabasco, “Dr. Desiderio G.
47
48 45 Carbajal Regional Hospital in Tabasco, Mexico and “Dr. Juan N. Navarro”
49
50 46 Psychiatric Hospital in Mexico City. Individuals used as controls (n=500) will not
51
52 47 have current or past history of any suicidal behavior, with no first degree relatives
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54 48 with a history of suicidal behavior and they will have to be unrelated to those in the
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3 49 case group. Both cases and controls will be interviewed by two psychiatrist or
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5 50 clinical specialists, together will determine the presence or absence of suicide
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7 51 attempt. All individuals will be Mexican with Mexican ascendancy of at least two
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9 52 generations (Mexican parents and grandparents); they will be recruited from
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11 53 several Mexican hospitals and outpatient clinics.
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17 54 18 *Patient and Public Involvement*

19 56 The outcomes of the present protocol will be directly communicated to the patients
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21 57 who participate or their legal caregivers. The results will also be discussed with
22
23 58 their psychiatrists and the corresponding health-education structures of the clinical
24
25 59 centers. However, the patients have not been directly participate in the study
26
27 60 design or any methodological procedures. Nevertheless, the authors of this
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29 61 protocol have clinical experience with SB patients and their families, which helped
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31 62 designed this study.
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37 63 38 *Ethics and dissemination*

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40 65 A written informed consent will be obtained from all individuals who accept
41
42 66 to participate. The study will be performed in accordance with the Helsinki
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44 67 declaration (59th General Assembly, Seoul, Korea, October 2008). This study was
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46 68 approved by the ethics and investigation commitments of the National Institute of
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48 69 Genomic Medicine on July 22nd 2015, No. CEI 215/13. We plan to disseminate
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50 70 research findings through scientific conferences and as a manuscript in peer-
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52 71 reviewed journals.
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3 73 *Clinical assessment*
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5 74 Diagnoses and clinical evaluations will be performed by at least two trained
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8 75 senior psychiatrists of whom at least one of them would have personally examined
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10 76 the patient. All participants will undergo semi-structured interviews that will include
11
12 77 life-time and family history of suicidal behavior, among other clinical features.
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14 78 Suicide attempt will be defined as a self-injurious act that had at least a partial
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16 79 intent to end one's life; the number of attempts, method used and medical damage
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19 80 of past suicide attempts will be obtained.
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24 82 *Genotyping*
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26 83 The DNA will be isolated from peripheral blood leukocytes samples using a
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28 84 standardized protocol of the Genomic Wizard Purification Kit from Promega, as
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30 85 previously reported ²²⁻²⁴. The integrity of genetic material will be checked on 1%
31
32 86 agarose gels and quantified by spectrophotometry using Nanodrop system.
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35 87 The genotyping will be performed using the Infinium PsychArray BeadChip,
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37 88 following the manufacturer's protocol ^{25 26}. This array contains approximately
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39 89 580000 genetic variants, wherein include a set of genetic variants previously
40
41 90 associated with a variety of psychiatric illnesses. All genotyping analyses will be
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43 91 performed at the National Institute of Genomic Medicine (INMEGEN). For quality
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45 92 controls, we will filter-out samples and variants with call rates lower than 98% and
46
47 93 variants deviating from Hardy-Weinberg equilibrium, with a chi-square p-value <
48
49 94 1×10^{-6} . The concordance between gender will be performed based on the
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51 95 heterozygosity of the X and Y chromosomes. All the filtering processes will be
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53 96 performed using the PLINK v1.9 software ²⁷. After that, we will perform a
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3 97 multidimensional scaling (MDS) analysis in PLINK, all SNPs that pass quality
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5 98 controls will be pruned and used to check population stratification in order to
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7 99 evaluate the ancestry of individuals included. After the MDS analysis, the first five
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9
10 100 components will be used as covariates in the association analysis. MDS
11
12 101 dimensions will be graphically represented using the “MDS-plot” option. After
13
14 102 quality control procedures, we will perform the imputation using the reference
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16 103 panels provided by the Haplotype Reference Consortium r.1.1; we will use the
17
18 104 Michigan Imputation Server for the imputation^{28 29}.

105

106 *Polygenic risk score calculation (PRS)*

107 PRS is a measurement of genetic liability to schizophrenia based on the
108 Psychiatric Genomics Consortium (PGC) schizophrenia GWAS³⁰. SNPs will be
109 selected to be included in the PRS calculation based on p values obtained in the
110 original PGC GWAS using PRSice³¹. PRS will be used to test an association with
111 suicide attempt using linear regression models adjusted by age, sex, and four
112 multidimensional-scaling components (MDS). The estimation of gene- or set-based
113 association tests using GWAS summary data, will be performed using Genome-
114 wide Complex Trait Analysis (GCTA)^{32 33}.

115

116 *Statistical analyses*

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

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3 121 mixed linear model analysis on imputed variants with a minor allele frequency of
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5 122 5%. The implementation of linear mixed models will be performed on the GCTA
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7 123 software³⁴. For the second workflow, will include a prediction of the deliriousness
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10 124 of variants using different prediction algorithms, such as PolyPhen, SIFT, CADD,
11
12 125 VEP and ENCODE³⁵⁻³⁹. After the prediction of the functional impact of the
13
14 126 variants, we will compare the cases allele frequency of loss-of-function, missense
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16 127 with deleterious effect, variants present in regulatory regions and variants with a
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18 128 PHRED-CADD score higher than 20, with the allele frequency of the populations
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20 129 reported in the 1000 genomes and ExAC database. GWAS analysis will be
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22 130 performed on cases with a history of suicide attempts and compared to non-suicide
23
24 131 attempters. Genome wide significance will be set at $P < 5 \times 10^{-08}$.
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30 31 *Power analysis calculation*

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33 134 For GWAS analysis, we performed power calculations in QUANTO 1.2.4
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35 135 (<http://biostats.usc.edu/software>)¹⁴. The analysis use a log additive model of
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37 136 inheritance to detect a power of 0.98 at significance threshold 5×10^{-08} to detect an
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39 137 effect size of $OR \geq 2$ with a MAF of 0.25 and a P_0 of 0.08. Also testing the lower
40
41 138 bounds of the effect sizes of the variants, we observe a power of 0.82 with an
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43 139 effect size of 1.8, $P_0=0.08$ and MAF=0.25 at the same significance level. The P_0
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45 140 that we used is the baseline risk of suicide attempt in our population based on
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47 141 previous reports^{40 41}. Therefore, our study will be powered to detect genetics
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49 142 effects.
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145 **Discussion**

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

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

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3 168 provide information to better comprehend the influence of the genetic background
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5 169 on the development of suicidal behavior, among psychiatric patients.
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7 170 Additionally, the findings of the present research could provide valuable information
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9 171 for future researches who attempt to identify genetic risk factors of suicidal
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11 172 behavior, and help detect and/or treat this disease. Nowadays, there are
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13 173 microarrays that have been used to study several genetic variables in Caucasian
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15 174 and Asian populations; but to our knowledge, there is no evidence reported of
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17 175 GWAS in studies that evaluate suicidal behavior in psychiatric patients. Therefore,
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19 176 the outcomes of the study derived of the current protocol, could provide essential
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21 177 information. The use of this type of genetic tools will allow us to identify associated
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23 178 SNPs, missense and insertions and indels in mental illnesses such as
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25 179 schizophrenia and bipolar disorder, as well as their possible participation as
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27 180 predictors of suicidal behavior in a Mexican population. Consequently, these
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29 181 findings could give important information to improve the design of future chips for
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31 182 molecular diagnosis of psychiatric disorders in Mexicans, which will be very useful
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33 183 in the prevention, diagnosis and prognosis of suicidal behavior in Mexico.
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40 184 In conclusion, the completion of the present protocol could impulse the
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42 185 design of a microarray that includes associated variants to SB in Mexican
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44 186 population. Moreover, the findings will give a better perspective of the participation
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46 187 of the genetic background as a predictor of suicidal behavior in psychiatric
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48 188 diseases. Hence, the outcomes would be useful in genetic research, as well as in
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50 189 prevention and early diagnosis of suicidal behavior in Mexicans
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3 191 **Ethics and disseminations:**
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5 192 The study will be conducted in compliance with local regulations and internationally
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7 193 established principles of the Declaration of Helsinki (59th General Assembly,
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9 194 Seoul, Korea, October 2008). This study was approved by the ethics and
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11 195 investigation commitments of the National Institute of Genomic Medicine on July
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13 196 22nd 2015, No. CEI 215/13. Before inclusion, all patients are required to sign an
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15 197 informed consent form.
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19 198 **Consent for publication:**
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21 199 Not applicable.
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23 200 **Availability of data and material:**
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25 201 Not applicable.
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28 202 **Competing interest statement:**
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30 203 The authors declare to have no competing interests.
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32

33 204 **Funding:**
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36
37 206 Humberto Nicolini.
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40 207 **Author contributions:**
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42 208 ADGM, HN and JJMM conceived the study, participated in its design, helped to
43
44 209 draft the manuscript and mentored TBGC. CATZ, ADGM and JJMM critically
45
46 210 revised successive drafts of the manuscript and provided important intellectual
47
48 211 input. CATZ and TBGC coordinated and supervised the integration of the
49
50 212 manuscript. ES, IEJR, HN and ADGM contributed to developing the analytic plan
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52 213 proposed for this study. All authors read and approved the final manuscript.
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216 The protocol study will be a doctoral thesis of TBGC.

217 References

- 218 1. Perlis RH, Huang J, Purcell S, et al. Genome-wide association study of suicide attempts in mood
219 disorder patients. *The American journal of psychiatry* 2010;167(12):1499-507. doi:
220 10.1176/appi.ajp.2010.10040541 [published Online First: 2010/11/03]
- 221 2. Pulay AJ, Rethelyi JM. Multimarker analysis suggests the involvement of BDNF signaling and
222 microRNA biosynthesis in suicidal behavior. *American journal of medical genetics Part B,*
223 *Neuropsychiatric genetics : the official publication of the International Society of*
224 *Psychiatric Genetics* 2016;171(6):763-76. doi: 10.1002/ajmg.b.32433 [published Online
225 First: 2016/02/28]
- 226 3. Mullins N, Perroud N, Uher R, et al. Genetic relationships between suicide attempts, suicidal
227 ideation and major psychiatric disorders: a genome-wide association and polygenic scoring
228 study. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official*
229 *publication of the International Society of Psychiatric Genetics* 2014;165B(5):428-37. doi:
230 10.1002/ajmg.b.32247 [published Online First: 2014/06/26]
- 231 4. Perroud N, Uher R, Ng MY, et al. Genome-wide association study of increasing suicidal ideation
232 during antidepressant treatment in the GENDEP project. *Pharmacogenomics J*
233 2012;12(1):68-77. doi: 10.1038/tpj.2010.70 [published Online First: 2010/09/30]
- 234 5. Stein MB, Ware EB, Mitchell C, et al. Genomewide association studies of suicide attempts in US
235 soldiers. *American journal of medical genetics Part B, Neuropsychiatric genetics : the*
236 *official publication of the International Society of Psychiatric Genetics* 2017;174(8):786-97.
237 doi: 10.1002/ajmg.b.32594 [published Online First: 2017/09/14]
- 238 6. Levine SZ, Goldberg Y, Yoffe R, et al. Suicide attempts in a national population of twins
239 concordant for psychoses. *European neuropsychopharmacology : the journal of the*
240 *European College of Neuropsychopharmacology* 2014;24(8):1203-9. doi:
241 10.1016/j.euroneuro.2014.05.014 [published Online First: 2014/06/28]
- 242 7. Linker J, Gillespie NA, Maes H, et al. Suicidal ideation, depression, and conduct disorder in a
243 sample of adolescent and young adult twins. *Suicide & life-threatening behavior*
244 2012;42(4):426-36. doi: 10.1111/j.1943-278X.2012.00101.x [published Online First:
245 2012/06/01]
- 246 8. Petersen L, Sorensen TI, Kragh Andersen P, et al. Genetic and familial environmental effects on
247 suicide attempts: a study of Danish adoptees and their biological and adoptive siblings.
248 *Journal of affective disorders* 2014;155:273-7. doi: 10.1016/j.jad.2013.11.012 [published
249 Online First: 2013/12/05]
- 250 9. Roy A, Rylander G, Sarchiapone M. Genetics of suicides. Family studies and molecular genetics.
251 *Annals of the New York Academy of Sciences* 1997;836:135-57. [published Online First:
252 1998/06/09]
- 253 10. Mirkovic B, Cohen D, Laurent C, et al. A case-control association study of 12 candidate genes
254 and attempted suicide in French adolescents. *International journal of adolescent medicine*
255 *and health* 2017 doi: 10.1515/ijamh-2017-0089 [published Online First: 2017/09/14]
- 256 11. Tombacz D, Maroti Z, Kalmar T, et al. High-Coverage Whole-Exome Sequencing Identifies
257 Candidate Genes for Suicide in Victims with Major Depressive Disorder. *Scientific reports*
258 2017;7(1):7106. doi: 10.1038/s41598-017-06522-3 [published Online First: 2017/08/05]

- 1
2
3 259 12. Sokolowski M, Wasserman J, Wasserman D. Rare CNVs in Suicide Attempt include
4 260 Schizophrenia-Associated Loci and Neurodevelopmental Genes: A Pilot Genome-Wide and
5 261 Family-Based Study. *PLoS one* 2016;11(12):e0168531. doi: 10.1371/journal.pone.0168531
6 262 [published Online First: 2016/12/29]
7
8 263 13. Gross JA, Bureau A, Croteau J, et al. A genome-wide copy number variant study of suicidal
9 264 behavior. *PLoS one* 2015;10(5):e0128369. doi: 10.1371/journal.pone.0128369 [published
10 265 Online First: 2015/05/27]
11 266 14. Galfalvy H, Haghghi F, Hodgkinson C, et al. A genome-wide association study of suicidal
12 267 behavior. *American journal of medical genetics Part B, Neuropsychiatric genetics : the*
13 268 *official publication of the International Society of Psychiatric Genetics* 2015;168(7):557-63.
14 269 doi: 10.1002/ajmg.b.32330 [published Online First: 2015/06/17]
15 270 15. Galfalvy H, Zalsman G, Huang YY, et al. A pilot genome wide association and gene expression
16 271 array study of suicide with and without major depression. *The world journal of biological*
17 272 *psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry*
18 273 2013;14(8):574-82. doi: 10.3109/15622975.2011.597875 [published Online First:
19 274 2011/11/09]
20
21 275 16. Laje G, Allen AS, Akula N, et al. Genome-wide association study of suicidal ideation emerging
22 276 during citalopram treatment of depressed outpatients. *Pharmacogenet Genomics*
23 277 2009;19(9):666-74. doi: 10.1097/FPC.0b013e32832e4bcd [published Online First:
24 278 2009/09/03]
25
26 279 17. Zai CC, Goncalves VF, Tiwari AK, et al. A genome-wide association study of suicide severity
27 280 scores in bipolar disorder. *Journal of psychiatric research* 2015;65:23-9. doi:
28 281 10.1016/j.jpsychires.2014.11.002 [published Online First: 2015/04/29]
29 282 18. Menke A, Domschke K, Czamara D, et al. Genome-wide association study of antidepressant
30 283 treatment-emergent suicidal ideation. *Neuropsychopharmacology : official publication of*
31 284 *the American College of Neuropsychopharmacology* 2012;37(3):797-807. doi:
32 285 10.1038/npp.2011.257 [published Online First: 2011/10/28]
33 286 19. Bani-Fatemi A, Graff A, Zai C, et al. GWAS analysis of suicide attempt in schizophrenia: Main
34 287 genetic effect and interaction with early life trauma. *Neurosci Lett* 2016;622:102-6. doi:
35 288 10.1016/j.neulet.2016.04.043 [published Online First: 2016/04/26]
36 289 20. Schosser A, Butler AW, Ising M, et al. Genomewide association scan of suicidal thoughts and
37 290 behaviour in major depression. *PLoS one* 2011;6(7):e20690. doi:
38 291 10.1371/journal.pone.0020690 [published Online First: 2011/07/14]
39 292 21. Willour VL, Seifuddin F, Mahon PB, et al. A genome-wide association study of attempted
40 293 suicide. *Molecular psychiatry* 2012;17(4):433-44. doi: 10.1038/mp.2011.4 [published
41 294 Online First: 2011/03/23]
42 295 22. Gonzalez-Castro TB, Nicolini H, Lanzagorta N, et al. The role of brain-derived neurotrophic
43 296 factor (BDNF) Val66Met genetic polymorphism in bipolar disorder: a case-control study,
44 297 comorbidities, and meta-analysis of 16,786 subjects. *Bipolar disorders* 2015;17(1):27-38.
45 298 doi: 10.1111/bdi.12227 [published Online First: 2014/07/22]
46 299 23. Gonzalez-Castro TB, Tovilla-Zarate C, Juarez-Rojop I, et al. Association of the 5HTR2A gene with
47 300 suicidal behavior: case-control study and updated meta-analysis. *BMC psychiatry*
48 301 2013;13:25. doi: 10.1186/1471-244x-13-25 [published Online First: 2013/01/15]
49 302 24. Gonzalez-Castro TB, Tovilla-Zarate CA, Juarez-Rojop I, et al. Association of 5HTR1A gene
50 303 variants with suicidal behavior: case-control study and updated meta-analysis. *Journal of*
51 304 *psychiatric research* 2013;47(11):1665-72. doi: 10.1016/j.jpsychires.2013.04.011
52 305 [published Online First: 2013/08/06]
53
54
55
56
57
58
59
60

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2
3 306 25. Guo Y, He J, Zhao S, et al. Illumina human exome genotyping array clustering and quality
4 307 control. *Nature protocols* 2014;9(11):2643-62. doi: 10.1038/nprot.2014.174 [published
5 308 Online First: 2014/10/17]
6 309 26. Borges G, Orozco R, Medina Mora ME. [Risk index for attempted suicide in Mexico]. *Salud*
7 310 *publica de Mexico* 2012;54(6):595-606. [published Online First: 2013/01/16]
8 311 27. Chang CC, Chow CC, Tellier LC, et al. Second-generation PLINK: rising to the challenge of larger
9 312 and richer datasets. *GigaScience* 2015;4:7. doi: 10.1186/s13742-015-0047-8 [published
10 313 Online First: 2015/02/28]
11 314 28. Loh PR, Danecek P, Palamara PF, et al. Reference-based phasing using the Haplotype Reference
12 315 Consortium panel. *Nat Genet* 2016;48(11):1443-48. doi: 10.1038/ng.3679 [published
13 316 Online First: 2016/10/28]
14 317 29. McCarthy S, Das S, Kretzschmar W, et al. A reference panel of 64,976 haplotypes for genotype
15 318 imputation. *Nat Genet* 2016;48(10):1279-83. doi: 10.1038/ng.3643 [published Online First:
16 319 2016/08/23]
17 320 30. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 2014;511(7510):421-
18 321 7. doi: 10.1038/nature13595 [published Online First: 2014/07/25]
19 322 31. Euesden J, Lewis CM, O'Reilly PF. PRSice: Polygenic Risk Score software. *Bioinformatics*
20 323 2015;31(9):1466-8. doi: 10.1093/bioinformatics/btu848 [published Online First:
21 324 2015/01/01]
22 325 32. Yang J, Lee SH, Goddard ME, et al. GCTA: a tool for genome-wide complex trait analysis. *Am J*
23 326 *Hum Genet* 2011;88(1):76-82. doi: 10.1016/j.ajhg.2010.11.011 [published Online First:
24 327 2010/12/21]
25 328 33. Bakshi A, Zhu Z, Vinkhuyzen AA, et al. Fast set-based association analysis using summary data
26 329 from GWAS identifies novel gene loci for human complex traits. *Sci Rep* 2016;6:32894. doi:
27 330 10.1038/srep32894 [published Online First: 2016/09/09]
28 331 34. Yang J, Lee SH, Goddard ME, et al. Genome-wide complex trait analysis (GCTA): methods, data
29 332 analyses, and interpretations. *Methods in molecular biology (Clifton, NJ)* 2013;1019:215-
30 333 36. doi: 10.1007/978-1-62703-447-0_9 [published Online First: 2013/06/13]
31 334 35. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations
32 335 using PolyPhen-2. *Current protocols in human genetics* 2013;Chapter 7:Unit7.20. doi:
33 336 10.1002/0471142905.hg0720s76 [published Online First: 2013/01/15]
34 337 36. Ng PC, Henikoff S. Predicting deleterious amino acid substitutions. *Genome research*
35 338 2001;11(5):863-74. doi: 10.1101/gr.176601 [published Online First: 2001/05/05]
36 339 37. McLaren W, Gil L, Hunt SE, et al. The Ensembl Variant Effect Predictor. *Genome biology*
37 340 2016;17(1):122. doi: 10.1186/s13059-016-0974-4 [published Online First: 2016/06/09]
38 341 38. An integrated encyclopedia of DNA elements in the human genome. *Nature*
39 342 2012;489(7414):57-74. doi: 10.1038/nature11247 [published Online First: 2012/09/08]
40 343 39. Kircher M, Witten DM, Jain P, et al. A general framework for estimating the relative
41 344 pathogenicity of human genetic variants. 2014;46(3):310-5. doi: 10.1038/ng.2892
42 345 40. Romero-Pimentel AL, Mendoza-Morales RC, Fresan A, et al. Demographic and Clinical
43 346 Characteristics of Completed Suicides in Mexico City 2014-2015. *Front Psychiatry*
44 347 2018;9:402. doi: 10.3389/fpsy.2018.00402 [published Online First: 2018/09/25]
45 348 41. Bachmann S. Epidemiology of Suicide and the Psychiatric Perspective. *International journal of*
46 349 *environmental research and public health* 2018;15(7) doi: 10.3390/ijerph15071425
47 350 [published Online First: 2018/07/11]
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BMJ Open

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

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Manuscripts

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3 **Genome-Wide Association Study of suicide attempt in a Mexican population:**
4 **A study protocol**
5

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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.

1 Introduction

2 Suicide is one of the leading causes of death worldwide. Individuals
3 diagnosed with a psychiatric disorder have higher rates of suicide compared to the
4 general population, which emphasizes a narrow relationship between suicide and
5 psychiatric disorders. In this line of evidence, a considerable majority of suicide
6 victims have had an undiagnosed psychiatric disorder at the time of death¹⁻³.
7 Furthermore, several studies consider psychiatric disorders as one of the main risk
8 factors of suicide. Unfortunately, up to today, the mechanisms that explain this
9 relationship have not been fully disentangled^{4 5}.

10 Suicidal behavior (SB), has been defined as a complex issue that results
11 from the combination of genetic variants along with personal experiences and
12 environmental contribution; altogether, these factors establish the disease
13 symptomatology manifestation ^{1 3 6}. The results of studies based on twins,
14 adoptions and families, support the heritability of SB, pinpointing a genetic
15 influence⁷⁻¹⁰. Subsequently, many candidate-gene association studies have been
16 performed, with the different phenotypes of SB: suicide attempts, suicide ideation
17 and accomplished suicide ^{1 3 6 11 12}. Unfortunately, SB is a polygenic trait and
18 candidate-gene association studies underestimate the genetic background. In an
19 attempt to search for this polygenic variation of SB, other strategies have been
20 developed, such as the genome-wide association studies (GWAS) ^{6 13 14}.

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

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3 25 predisposition to SB has been proposed and GWAS have suggested candidate loci
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5 26 or pathways, the only information available comes from studies analyzing
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7 27 Caucasians or Asians populations, leaving behind Latin American populations
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9 28 including Mexicans¹⁷⁻²¹. Therefore, more studies are necessary in order to have a
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11 29 better comprehension of the genetic background of SB.
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16 17 31 Objective

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19 32 Our aim is to perform the first genome-wide association study of suicide
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21 33 attempters in a Mexican population, in order to explore and define the involvement
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23 34 of a genetic diathesis that predisposes to SB in this population. In addition, we will
24
25 35 explore the hypothesis that there are genes and genetic variants that increase the
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27 36 risk of suicide attempt in Mexican population and these factors could be common in
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29 37 individuals with a psychiatric diagnostic.
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35 39 **Methods and analysis**

36 37 40 *Sample population and setting*

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40 41 The case group (n=700) will be formed by individuals who have had at least one
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42 42 suicide attempt, and this will be determined using Structured Clinical Interview for
43
44 43 DSM-IV (SCID-I and II) in the Psychiatry out-patient area from three clinical
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46 44 centers: “Dr. Gustavo A. Roviroso” General Hospital in Tabasco, “Dr. Desiderio G.
47
48 45 Carbajal Regional Hospital in Tabasco, Mexico and “Dr. Juan N. Navarro”
49
50 46 Psychiatric Hospital in Mexico City. Individuals used as controls (n=500) will not
51
52 47 have current or past history of any suicidal behavior, with no first degree relatives
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54 48 with a history of suicidal behavior and they will have to be unrelated to those in the
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3 49 case group. Both cases and controls will be interviewed by two psychiatrist or
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5 50 clinical specialists, together will determine the presence or absence of suicide
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7 51 attempt. All individuals will be Mexican with Mexican ascendancy of at least two
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9 52 generations (Mexican parents and grandparents); they will be recruited from
10
11 53 several Mexican hospitals and outpatient clinics.
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17 54 18 *Patient and Public Involvement*

19 56 The outcomes of the present protocol will be directly communicated to the patients
20
21 57 who participate or their legal caregivers. The results will also be discussed with
22
23 58 their psychiatrists and the corresponding health-education structures of the clinical
24
25 59 centers. However, the patients have not been directly participate in the study
26
27 60 design or any methodological procedures. Nevertheless, the authors of this
28
29 61 protocol have clinical experience with SB patients and their families, which helped
30
31 62 designed this study.
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37 63 38 *Ethics and dissemination*

39 64
40 65 A written informed consent will be obtained from all individuals who accept
41
42 66 to participate. The study will be performed in accordance with the Helsinki
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44 67 declaration (59th General Assembly, Seoul, Korea, October 2008). This study was
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46 68 approved by the ethics and investigation commitments of the National Institute of
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48 69 Genomic Medicine on July 22nd 2015, No. CEI 215/13. We plan to disseminate
49
50 70 research findings through scientific conferences and as a manuscript in peer-
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52 71 reviewed journals.
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3 73 *Clinical assessment*
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5 74 Diagnoses and clinical evaluations will be performed by at least two trained
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8 75 senior psychiatrists of whom at least one of them would have personally examined
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10 76 the patient. All participants will undergo semi-structured interviews that will include
11
12 77 life-time and family history of suicidal behavior, among other clinical features.
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14 78 Suicide attempt will be defined as a self-injurious act that had at least a partial
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17 79 intent to end one's life; the number of attempts, method used and medical damage
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19 80 of past suicide attempts will be obtained.
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24 82 *Genotyping*
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26 83 The DNA will be isolated from peripheral blood leukocytes samples using a
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28 84 standardized protocol of the Genomic Wizard Purification Kit from Promega, as
29
30 85 previously reported²²⁻²⁴. The integrity of genetic material will be checked on 1%
31
32 86 agarose gels and quantified by spectrophotometry using Nanodrop system.
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35 87 The genotyping will be performed using the Infinium PsychArray BeadChip,
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37 88 following the manufacturer's protocol ^{25 26}. This array contains approximately
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39 89 580000 genetic variants, wherein include a set of genetic variants previously
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41
42 90 associated with a variety of psychiatric illnesses. All genotyping analyses will be
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44 91 performed at the National Institute of Genomic Medicine (INMEGEN). For quality
45
46 92 controls, we will filter-out samples and variants with call rates lower than 98% and
47
48 93 variants deviating from Hardy-Weinberg equilibrium, with a chi-square p-value <
49
50 94 1×10^{-6} . The concordance between gender will be performed based on the
51
52 95 heterozygosity of the X and Y chromosomes. All the filtering processes will be
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54 96 performed using the PLINK v1.9 software ²⁷. After that, we will perform a
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3 97 multidimensional scaling (MDS) analysis in PLINK, all SNPs that pass quality
4
5 98 controls will be pruned and used to check population stratification in order to
6
7 99 evaluate the ancestry of individuals included. After the MDS analysis, the first five
8
9
10 100 components will be used as covariates in the association analysis. MDS
11
12 101 dimensions will be graphically represented using the “MDS-plot” option. After
13
14 102 quality control procedures, we will perform the imputation using the reference
15
16 103 panels provided by the Haplotype Reference Consortium r.1.1; we will use the
17
18 104 Michigan Imputation Server for the imputation^{28 29}.

105

106 *Polygenic risk score calculation (PRS)*

107 PRS is a measurement of genetic liability to schizophrenia based on the
108 Psychiatric Genomics Consortium (PGC) schizophrenia GWAS³⁰. SNPs will be
109 selected to be included in the PRS calculation based on p values obtained in the
110 original PGC GWAS using PRSice³¹. PRS will be used to test an association with
111 suicide attempt using linear regression models adjusted by age, sex, and four
112 multidimensional-scaling components (MDS). The estimation of gene- or set-based
113 association tests using GWAS summary data, will be performed using Genome-
114 wide Complex Trait Analysis (GCTA)^{32 33}.

115

116 *Statistical analyses*

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

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3 121 mixed linear model analysis on imputed variants with a minor allele frequency of
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5 122 5%. The implementation of linear mixed models will be performed on the GCTA
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7 123 software³⁴. For the second workflow, will include a prediction of the deliriousness
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9
10 124 of variants using different prediction algorithms, such as PolyPhen, SIFT, CADD,
11
12 125 VEP and ENCODE³⁵⁻³⁹. After the prediction of the functional impact of the
13
14 126 variants, we will compare the cases allele frequency of loss-of-function, missense
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16 127 with deleterious effect, variants present in regulatory regions and variants with a
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18 128 PHRED-CADD score higher than 20, with the allele frequency of the populations
19
20 129 reported in the 1000 genomes and ExAC database. GWAS analysis will be
21
22 130 performed on cases with a history of suicide attempts and compared to non-suicide
23
24 131 attempters. Genome wide significance will be set at $P < 5 \times 10^{-08}$.
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133 *Power analysis calculation*

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33 134 For GWAS analysis, we performed power calculations in QUANTO 1.2.4
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35 135 (<http://biostats.usc.edu/software>). The analysis uses a log additive model of
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37 136 inheritance to detect a power of 0.82 at significance threshold 5×10^{-08} to detect an
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39 137 effect size of $OR \geq 1.8$ with a P_0 of 0.08 and MAF of 0.25, as in a previous study¹⁴.
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41 138 The P_0 that we used is the baseline risk of suicide attempt in our population based
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43 139 on previous reports^{40 41}. Therefore, our study will be powered to detect genetics
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45 140 effects.
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145 **Discussion**

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

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

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3 168 provide information to better comprehend the influence of the genetic background
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5 169 on the development of suicidal behavior, among psychiatric patients.
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7 170 Additionally, the findings of the present research could provide valuable information
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9 171 for future researches who attempt to identify genetic risk factors of suicidal
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11 172 behavior, and help detect and/or treat this disease. Nowadays, there are
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13 173 microarrays that have been used to study several genetic variables in Caucasian
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15 174 and Asian populations; but to our knowledge, there is no evidence reported of
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17 175 GWAS in studies that evaluate suicidal behavior in psychiatric patients. Therefore,
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19 176 the outcomes of the study derived of the current protocol, could provide essential
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21 177 information. The use of this type of genetic tools will allow us to identify associated
22
23 178 SNPs, missense and insertions and indels in mental illnesses such as
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25 179 schizophrenia and bipolar disorder, as well as their possible participation as
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27 180 predictors of suicidal behavior in a Mexican population. Consequently, these
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29 181 findings could give important information to improve the design of future chips for
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31 182 molecular diagnosis of psychiatric disorders in Mexicans, which will be very useful
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33 183 in the prevention, diagnosis and prognosis of suicidal behavior in Mexico.
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40 184 In conclusion, the completion of the present protocol could impulse the
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42 185 design of a microarray that includes associated variants to SB in Mexican
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44 186 population. Moreover, the findings will give a better perspective of the participation
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46 187 of the genetic background as a predictor of suicidal behavior in psychiatric
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48 188 diseases. Hence, the outcomes would be useful in genetic research, as well as in
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50 189 prevention and early diagnosis of suicidal behavior in Mexicans
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3 191 **Ethics and disseminations:**
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5 192 The study will be conducted in compliance with local regulations and internationally
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7 193 established principles of the Declaration of Helsinki (59th General Assembly,
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9 194 Seoul, Korea, October 2008). This study was approved by the ethics and
10
11 195 investigation commitments of the National Institute of Genomic Medicine on July
12
13 196 22nd 2015, No. CEI 215/13. Before inclusion, all patients are required to sign an
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15 197 informed consent form.
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21 199 **Consent for publication:**
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23 200 Not applicable.
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28 202 **Availability of data and material:**
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30 203 Not applicable.
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35 205 **Competing interest statement:**
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37 206 The authors declare to have no competing interests.
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46 210 Humberto Nicolini.
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50
51 212 **Author contributions:**
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53 213 ADGM, HN and JJMM conceived the study, participated in its design, helped to
54
55 214 draft the manuscript and mentored TBGC. CATZ, ADGM and JJMM critically
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3 215 revised successive drafts of the manuscript and provided important intellectual
4
5 216 input. CATZ and TBGC coordinated and supervised the integration of the
6
7 217 manuscript. ES, IEJR, HN and ADGM contributed to developing the analytic plan
8
9 218 proposed for this study. All authors read and approved the final manuscript.
10
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12 219

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16
17 221 The protocol study will be a doctoral thesis of TBGC.
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239 **References**

- 240 1. Perlis RH, Huang J, Purcell S, et al. Genome-wide association study of suicide attempts in mood
241 disorder patients. *The American journal of psychiatry* 2010;167(12):1499-507. doi:
242 10.1176/appi.ajp.2010.10040541 [published Online First: 2010/11/03]
- 243 2. Pulay AJ, Rethelyi JM. Multimarker analysis suggests the involvement of BDNF signaling and
244 microRNA biosynthesis in suicidal behavior. *American journal of medical genetics Part B,*
245 *Neuropsychiatric genetics : the official publication of the International Society of*
246 *Psychiatric Genetics* 2016;171(6):763-76. doi: 10.1002/ajmg.b.32433 [published Online
247 First: 2016/02/28]
- 248 3. Mullins N, Perroud N, Uher R, et al. Genetic relationships between suicide attempts, suicidal
249 ideation and major psychiatric disorders: a genome-wide association and polygenic scoring
250 study. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official*
251 *publication of the International Society of Psychiatric Genetics* 2014;165B(5):428-37. doi:
252 10.1002/ajmg.b.32247 [published Online First: 2014/06/26]
- 253 4. Perroud N, Uher R, Ng MY, et al. Genome-wide association study of increasing suicidal ideation
254 during antidepressant treatment in the GENDEP project. *Pharmacogenomics J*
255 2012;12(1):68-77. doi: 10.1038/tpj.2010.70 [published Online First: 2010/09/30]
- 256 5. Stein MB, Ware EB, Mitchell C, et al. Genomewide association studies of suicide attempts in US
257 soldiers. *American journal of medical genetics Part B, Neuropsychiatric genetics : the*
258 *official publication of the International Society of Psychiatric Genetics* 2017;174(8):786-97.
259 doi: 10.1002/ajmg.b.32594 [published Online First: 2017/09/14]
- 260 6. Sokolowski M, Wasserman J, Wasserman D. Rare CNVs in Suicide Attempt include
261 Schizophrenia-Associated Loci and Neurodevelopmental Genes: A Pilot Genome-Wide and
262 Family-Based Study. *PloS one* 2016;11(12):e0168531. doi: 10.1371/journal.pone.0168531
263 [published Online First: 2016/12/29]
- 264 7. Levine SZ, Goldberg Y, Yoffe R, et al. Suicide attempts in a national population of twins
265 concordant for psychoses. *European neuropsychopharmacology : the journal of the*
266 *European College of Neuropsychopharmacology* 2014;24(8):1203-9. doi:
267 10.1016/j.euroneuro.2014.05.014 [published Online First: 2014/06/28]
- 268 8. Linker J, Gillespie NA, Maes H, et al. Suicidal ideation, depression, and conduct disorder in a
269 sample of adolescent and young adult twins. *Suicide & life-threatening behavior*
270 2012;42(4):426-36. doi: 10.1111/j.1943-278X.2012.00101.x [published Online First:
271 2012/06/01]
- 272 9. Petersen L, Sorensen TI, Kragh Andersen P, et al. Genetic and familial environmental effects on
273 suicide attempts: a study of Danish adoptees and their biological and adoptive siblings.
274 *Journal of affective disorders* 2014;155:273-7. doi: 10.1016/j.jad.2013.11.012 [published
275 Online First: 2013/12/05]
- 276 10. Roy A, Rylander G, Sarchiapone M. Genetics of suicides. Family studies and molecular genetics.
277 *Annals of the New York Academy of Sciences* 1997;836:135-57. [published Online First:
278 1998/06/09]
- 279 11. Mirkovic B, Cohen D, Laurent C, et al. A case-control association study of 12 candidate genes
280 and attempted suicide in French adolescents. *International journal of adolescent medicine*
281 *and health* 2017 doi: 10.1515/ijamh-2017-0089 [published Online First: 2017/09/14]
- 282 12. Tombacz D, Maroti Z, Kalmar T, et al. High-Coverage Whole-Exome Sequencing Identifies
283 Candidate Genes for Suicide in Victims with Major Depressive Disorder. *Scientific reports*
284 2017;7(1):7106. doi: 10.1038/s41598-017-06522-3 [published Online First: 2017/08/05]

- 1
2
3 285 13. Gross JA, Bureau A, Croteau J, et al. A genome-wide copy number variant study of suicidal
4 286 behavior. *PLoS one* 2015;10(5):e0128369. doi: 10.1371/journal.pone.0128369 [published
5 287 Online First: 2015/05/27]
6 288 14. Galfalvy H, Haghghi F, Hodgkinson C, et al. A genome-wide association study of suicidal
7 289 behavior. *American journal of medical genetics Part B, Neuropsychiatric genetics : the*
8 290 *official publication of the International Society of Psychiatric Genetics* 2015;168(7):557-63.
9 291 doi: 10.1002/ajmg.b.32330 [published Online First: 2015/06/17]
10 292 15. Galfalvy H, Zalsman G, Huang YY, et al. A pilot genome wide association and gene expression
11 293 array study of suicide with and without major depression. *The world journal of biological*
12 294 *psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry*
13 295 2013;14(8):574-82. doi: 10.3109/15622975.2011.597875 [published Online First:
14 296 2011/11/09]
15 297 16. Laje G, Allen AS, Akula N, et al. Genome-wide association study of suicidal ideation emerging
16 298 during citalopram treatment of depressed outpatients. *Pharmacogenet Genomics*
17 299 2009;19(9):666-74. doi: 10.1097/FPC.0b013e32832e4bcd [published Online First:
18 300 2009/09/03]
19 301 17. Zai CC, Goncalves VF, Tiwari AK, et al. A genome-wide association study of suicide severity
20 302 scores in bipolar disorder. *Journal of psychiatric research* 2015;65:23-9. doi:
21 303 10.1016/j.jpsychires.2014.11.002 [published Online First: 2015/04/29]
22 304 18. Menke A, Domschke K, Czamara D, et al. Genome-wide association study of antidepressant
23 305 treatment-emergent suicidal ideation. *Neuropsychopharmacology : official publication of*
24 306 *the American College of Neuropsychopharmacology* 2012;37(3):797-807. doi:
25 307 10.1038/npp.2011.257 [published Online First: 2011/10/28]
26 308 19. Bani-Fatemi A, Graff A, Zai C, et al. GWAS analysis of suicide attempt in schizophrenia: Main
27 309 genetic effect and interaction with early life trauma. *Neurosci Lett* 2016;622:102-6. doi:
28 310 10.1016/j.neulet.2016.04.043 [published Online First: 2016/04/26]
29 311 20. Schosser A, Butler AW, Ising M, et al. Genomewide association scan of suicidal thoughts and
30 312 behaviour in major depression. *PLoS one* 2011;6(7):e20690. doi:
31 313 10.1371/journal.pone.0020690 [published Online First: 2011/07/14]
32 314 21. Willour VL, Seifuddin F, Mahon PB, et al. A genome-wide association study of attempted
33 315 suicide. *Molecular psychiatry* 2012;17(4):433-44. doi: 10.1038/mp.2011.4 [published
34 316 Online First: 2011/03/23]
35 317 22. Gonzalez-Castro TB, Nicolini H, Lanzagorta N, et al. The role of brain-derived neurotrophic
36 318 factor (BDNF) Val66Met genetic polymorphism in bipolar disorder: a case-control study,
37 319 comorbidities, and meta-analysis of 16,786 subjects. *Bipolar disorders* 2015;17(1):27-38.
38 320 doi: 10.1111/bdi.12227 [published Online First: 2014/07/22]
39 321 23. Gonzalez-Castro TB, Tovilla-Zarate C, Juarez-Rojop I, et al. Association of the 5HTR2A gene with
40 322 suicidal behavior: case-control study and updated meta-analysis. *BMC psychiatry*
41 323 2013;13:25. doi: 10.1186/1471-244x-13-25 [published Online First: 2013/01/15]
42 324 24. Gonzalez-Castro TB, Tovilla-Zarate CA, Juarez-Rojop I, et al. Association of 5HTR1A gene
43 325 variants with suicidal behavior: case-control study and updated meta-analysis. *Journal of*
44 326 *psychiatric research* 2013;47(11):1665-72. doi: 10.1016/j.jpsychires.2013.04.011
45 327 [published Online First: 2013/08/06]
46 328 25. Guo Y, He J, Zhao S, et al. Illumina human exome genotyping array clustering and quality
47 329 control. *Nature protocols* 2014;9(11):2643-62. doi: 10.1038/nprot.2014.174 [published
48 330 Online First: 2014/10/17]
49 331 26. Borges G, Orozco R, Medina Mora ME. [Risk index for attempted suicide in Mexico]. *Salud*
50 332 *publica de Mexico* 2012;54(6):595-606. [published Online First: 2013/01/16]
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3 333 27. Chang CC, Chow CC, Tellier LC, et al. Second-generation PLINK: rising to the challenge of larger
4 334 and richer datasets. *GigaScience* 2015;4:7. doi: 10.1186/s13742-015-0047-8 [published
5 335 Online First: 2015/02/28]
6 336 28. Loh PR, Danecek P, Palamara PF, et al. Reference-based phasing using the Haplotype Reference
7 337 Consortium panel. *Nat Genet* 2016;48(11):1443-48. doi: 10.1038/ng.3679 [published
8 338 Online First: 2016/10/28]
9 339 29. McCarthy S, Das S, Kretzschmar W, et al. A reference panel of 64,976 haplotypes for genotype
10 340 imputation. *Nat Genet* 2016;48(10):1279-83. doi: 10.1038/ng.3643 [published Online First:
11 341 2016/08/23]
12 342 30. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 2014;511(7510):421-
13 343 7. doi: 10.1038/nature13595 [published Online First: 2014/07/25]
14 344 31. Euesden J, Lewis CM, O'Reilly PF. PRSice: Polygenic Risk Score software. *Bioinformatics*
15 345 2015;31(9):1466-8. doi: 10.1093/bioinformatics/btu848 [published Online First:
16 346 2015/01/01]
17 347 32. Yang J, Lee SH, Goddard ME, et al. GCTA: a tool for genome-wide complex trait analysis. *Am J*
18 348 *Hum Genet* 2011;88(1):76-82. doi: 10.1016/j.ajhg.2010.11.011 [published Online First:
19 349 2010/12/21]
20 350 33. Bakshi A, Zhu Z, Vinkhuyzen AA, et al. Fast set-based association analysis using summary data
21 351 from GWAS identifies novel gene loci for human complex traits. *Sci Rep* 2016;6:32894. doi:
22 352 10.1038/srep32894 [published Online First: 2016/09/09]
23 353 34. Yang J, Lee SH, Goddard ME, et al. Genome-wide complex trait analysis (GCTA): methods, data
24 354 analyses, and interpretations. *Methods in molecular biology (Clifton, NJ)* 2013;1019:215-
25 355 36. doi: 10.1007/978-1-62703-447-0_9 [published Online First: 2013/06/13]
26 356 35. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations
27 357 using PolyPhen-2. *Current protocols in human genetics* 2013;Chapter 7:Unit7.20. doi:
28 358 10.1002/0471142905.hg0720s76 [published Online First: 2013/01/15]
29 359 36. Ng PC, Henikoff S. Predicting deleterious amino acid substitutions. *Genome research*
30 360 2001;11(5):863-74. doi: 10.1101/gr.176601 [published Online First: 2001/05/05]
31 361 37. McLaren W, Gil L, Hunt SE, et al. The Ensembl Variant Effect Predictor. *Genome biology*
32 362 2016;17(1):122. doi: 10.1186/s13059-016-0974-4 [published Online First: 2016/06/09]
33 363 38. An integrated encyclopedia of DNA elements in the human genome. *Nature*
34 364 2012;489(7414):57-74. doi: 10.1038/nature11247 [published Online First: 2012/09/08]
35 365 39. Kircher M, Witten DM, Jain P, et al. A general framework for estimating the relative
36 366 pathogenicity of human genetic variants. 2014;46(3):310-5. doi: 10.1038/ng.2892
37 367 40. Romero-Pimentel AL, Mendoza-Morales RC, Fresan A, et al. Demographic and Clinical
38 368 Characteristics of Completed Suicides in Mexico City 2014-2015. *Front Psychiatry*
39 369 2018;9:402. doi: 10.3389/fpsy.2018.00402 [published Online First: 2018/09/25]
40 370 41. Bachmann S. Epidemiology of Suicide and the Psychiatric Perspective. *International journal of*
41 371 *environmental research and public health* 2018;15(7) doi: 10.3390/ijerph15071425
42 372 [published Online First: 2018/07/11]
43 373

BMJ Open

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

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Keywords:	Suicide & self-harm < PSYCHIATRY, MENTAL HEALTH, Schizophrenia & psychotic disorders < PSYCHIATRY, GENETICS

SCHOLARONE™
Manuscripts

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3 **Genome-Wide Association Study of suicide attempt in a Mexican population:**
4 **A study protocol**
5

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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.

1 Introduction

2 Suicide is one of the leading causes of death worldwide. Individuals
3 diagnosed with psychiatric disorders have higher rates of suicide compared with the
4 general population, which emphasizes a narrow relationship between suicide and
5 psychiatric disorders. In this line of evidence, a considerable majority of suicide
6 victims have had an undiagnosed psychiatric disorder at the time of death¹⁻³.
7 Furthermore, several studies consider psychiatric disorders as one of the main risk
8 factors of suicide. Unfortunately, up to today, the mechanisms of this relationship
9 have not been fully disentangled^{4 5}.

10 Suicidal behavior (SB), has been defined as a complex issue that results from
11 the combination of genetic variants along with personal experiences and
12 environmental contribution; altogether, these factors establish the disease
13 symptomatology manifestation^{1 3 6}. The results of studies based on twins, adoptions
14 and families, support the heritability of SB, pinpointing a genetic influence⁷⁻¹⁰.
15 Subsequently, many candidate-gene association studies have been performed,
16 studying the different phenotypes of SB: suicide attempts, suicide ideation and
17 accomplished suicide^{1 3 6 11 12}. Unfortunately, SB is a polygenic trait and candidate-
18 gene association studies underestimate the genetic background. In an attempt to
19 search for this polygenic variation of SB, other strategies have been developed, such
20 as the genome-wide association studies (GWAS)^{6 13 14}.

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

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3 25 predisposition has been proposed and GWAS have suggested candidate loci or
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5 26 pathways, the only information available comes from studies analyzing Caucasian
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7 27 or Asian populations, leaving behind Latin American populations including
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9 28 Mexicans¹⁷⁻²¹. Therefore, more studies are necessary to have a better
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11 29 comprehension of the SB genetic background.
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16 17 31 Objectives

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19 32 Our aim is to perform the first genome-wide association study of suicide
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21 33 attempters in a Mexican population, in order to explore and define the involvement
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23 34 of a genetic diathesis that predisposes to SB in this population. In addition, we will
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25 35 explore the hypothesis that there are genes and genetic variants that increase the
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27 36 risk of suicide attempt in Mexican population and these factors could be common in
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29 37 individuals with a psychiatric diagnostic.
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35 39 **Methods and analysis**

36 37 40 *Sample population and setting*

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40 41 The case group (n=700) will be formed by individuals who have had at least one
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42 42 suicide attempt, and this will be determined using the Structured Clinical Interview
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44 43 for DSM-IV (SCID-I and II) in Psychiatry out-patient areas from three clinical centers:
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46 44 “Dr. Gustavo A. Rovirosa” General Hospital in Tabasco, “Dr. Desiderio G. Carbajal
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48 45 Regional Hospital in Tabasco, Mexico and “Dr. Juan N. Navarro” Psychiatric Hospital
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50 46 in Mexico City. Individuals used as controls (n=500) will not have current or past
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52 47 history of any suicidal behavior, will not have first degree relatives with suicidal
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54 48 behavior history and they will be unrelated to those in the case group. Both cases
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3 49 and controls will be interviewed by two psychiatrist or clinical specialists, together
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5 50 they will determine the presence or absence of suicide attempt. All individuals will
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7 51 be Mexicans with Mexican ascendancy of at least two generations (Mexican parents
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9 52 and grandparents); they will be recruited from several hospitals and outpatient clinics
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11 53 in Mexico.
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16 17 54 18 19 55 *Patient and Public Involvement*

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21 56 The outcomes of the present protocol will be directly communicated to patients who
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23 57 participate or their legal caregivers. The results will also be discussed with their
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25 58 psychiatrists and the corresponding health-education structures of the clinical
26
27 59 centers. However, the patients have not directly participated in the study design or
28
29 60 any methodological procedures.
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33 61 34 62 *Ethics and dissemination*

35 63 A written informed consent will be obtained from all individuals who accept to
36
37 64 participate. The study will be performed in accordance with the Helsinki declaration
38
39 65 (59th General Assembly, Seoul, Korea, October 2008). This study has been already
40
41 66 approved by the ethics and investigation committees of the National Institute of
42
43 67 Genomic Medicine on July 22nd 2015, No. CEI 215/13. We plan to disseminate
44
45 68 research findings in scientific conferences and as a manuscript in peer-reviewed
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47 69 journals.
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51 70 52 53 71 *Clinical assessment*

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3 72 Diagnoses and clinical evaluations will be performed by at least two trained
4
5 73 senior psychiatrists of whom at least one of them would have personally examined
6
7 74 the patient. All participants will undergo semi-structured interviews that will include
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9
10 75 life-time and family history of suicidal behavior, among other clinical features. Suicide
11
12 76 attempt will be defined as a self-injurious act that had at least a partial intent to end
13
14 77 one's life; the number of attempts, method used and medical damage of past suicide
15
16 78 attempts will be gathered.
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20 21 80 *Genotyping*

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24 81 DNA will be isolated from peripheral blood leukocytes samples using a
25
26 82 standardized protocol of the Genomic Wizard Purification Kit from Promega, as
27
28 83 previously reported²²⁻²⁴. The integrity of genetic material will be checked on 1%
29
30 84 agarose gels and quantified by spectrophotometry using the Nanodrop system.
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34
35 86 The genotyping will be performed using the Infinium PsychArray BeadChip,
36
37 87 following the manufacturer's protocol^{25 26}. This array contains approximately 580000
38
39 88 genetic variants, wherein include a set of genetic variants previously associated with
40
41
42 89 a variety of psychiatric illnesses. All genotyping analyses will be performed at the
43
44 90 National Institute of Genomic Medicine (INMEGEN). For quality controls, we will
45
46 91 filter-out samples and variants with call rates lower than 98% and variants deviating
47
48 92 from Hardy-Weinberg equilibrium, with a chi-square p-value $< 1 \times 10^{-6}$. Gender
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50 93 concordance will be performed based on heterozygosity of X and Y chromosomes.
51
52 94 All filtering processes will be done using the PLINK v1.9 software²⁷. Then, we will
53
54 95 perform a multidimensional scaling (MDS) analysis in PLINK, all SNPs that pass
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3 96 quality controls will be pruned and used to check population stratification in order to
4
5 97 evaluate the ancestry of the individuals included. After the MDS analysis, the first
6
7 98 five components will be used as covariates in the association analysis. MDS
8
9
10 99 dimensions will be graphically represented using the “MDS-plot” option. After quality
11
12 100 control procedures, we will perform the imputation using reference panels provided
13
14 101 by the Haplotype Reference Consortium r.1.1; we will use the Michigan Imputation
15
16
17 102 Server for the imputation^{28 29}.

18
19 103

20 21 104 *Polygenic risk score calculation (PRS)*

22
23 105 PRS is a measurement of genetic liability to schizophrenia, based on the
24
25 106 Psychiatric Genomics Consortium (PGC) schizophrenia GWAS³⁰. SNPs will be
26
27
28 107 selected and used in the PRS calculation based on p values obtained in the original
29
30 108 PGC GWAS using PRSice³¹. PRS will be performed to search for suicide attempt
31
32
33 109 associations using linear regression models adjusted by age, sex, and four
34
35 110 multidimensional-scaling components (MDS). The estimation of gene- or set-based
36
37 111 association tests using GWAS summary data, will be performed using Genome-wide
38
39 112 Complex Trait Analysis (GCTA)^{32 33}.

40
41 113

42 43 114 *Statistical analyses*

44
45 115 In order to evaluate the effect of genetic variants on suicidal behavior, we will
46
47 116 perform two workflows: A) a classic GWAS analysis applying the same importance
48
49 117 to all the variants and B) algorithms for predicting deleteriousness of variants.
50
51 118 Concerning the first workflow, we will conduct a mixed linear model analysis on
52
53
54 119 imputed variants with a minor allele frequency of 5%. The implementation of linear
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3 120 mixed models will be performed using the GCTA software ³⁴. For the second
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5 121 workflow, we will include the prediction of deliriousness of variants using different
6
7 122 prediction algorithms, such as PolyPhen, SIFT, CADD, VEP and ENCODE ³⁵⁻³⁹.
8
9
10 123 After the functional impact on variants prediction, we will compare cases' allele
11
12 124 frequency of loss-of-function, missense with deleterious effect, variants present in
13
14 125 regulatory regions and variants with a PHRED-CADD score higher than 20, with the
15
16 126 allele frequency of populations reported in the 1000 genomes and ExAC database.
17
18
19 127 GWAS analysis will be performed on cases with history of suicide attempt and
20
21 128 compared with non-suicide attempters. Genome wide significance will be set at P
22
23
24 129 $<5 \times 10^{-08}$.

130

131 *Power analysis calculation*

132 For the GWAS analysis, we will perform power calculations using QUANTO 1.2.4
133 (<http://biostats.usc.edu/software>). This analysis uses a log additive model of
134 inheritance and is capable of detecting a power of 0.82 at significance threshold
135 5×10^{-08} to detect an effect size of OR ≥ 1.8 with a P_0 of 0.08 and MAF of 0.25, as
136 observed in a previous study¹⁴. The P_0 that we will use is the baseline risk of suicide
137 attempt in our population based on previous reports ^{40 41}. Therefore, our study will
138 be powered to detect genetics effects.

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140 **Discussion**

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

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3 144 predisposition to manifest SB has been supported by several investigations, but the
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5 145 understanding of the precise genetic system that causes such vulnerability to
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7 146 suicidal tendencies is still largely incomplete. Hence, the principal aim of our protocol
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10 147 study is to explore the potential genetic influence on SB in a Mexican population,
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12 148 throughout a genome-wide association study. In addition, we want to emphasize that
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14 149 to our knowledge, this study protocol will be the first one to evaluate suicidal behavior
15
16
17 150 in patients with schizophrenia and bipolar disorder in a Mexican population.

18
19 151 The majority of genetic epidemiology evidence suggest that suicidal behavior
20
21 152 is a complex issue, where there are multiple genes that have a small effect over SB;
22
23 153 but if combined, could become predisposing factors. Therefore, association studies
24
25 154 that detect small effect contributions can be more useful, which is a strength of this
26
27 155 protocol. In this sense, one of the most powerful strengths of the GWAS is that it
28
29 156 uses many genetic markers across the whole genome to search for associations
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31 157 with a particular disease; as it is based on no prior assumptions, it explores a large
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33 158 number of genetic variants. For these reasons, we will use GWAS's results to
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35 159 explore the genetic influence on suicidal behavior in schizophrenics with and without
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37 160 SA, bipolar patients with and without SA, suicide attempters and healthy subjects as
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39 161 controls. Therefore, the results of this study will provide information to better
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41 162 comprehend the influence of the genetic background when developing suicidal
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43 163 behavior, among psychiatric patients.

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45 164 Additionally, the findings of the present research could provide valuable information
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47 165 for future researchers who attempt to identify genetic risk factors of suicidal behavior,
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49 166 and help detect and/or treat this disease. Nowadays, there are microarrays that have
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51 167 been used to study several genetic variables in Caucasian and Asian populations;
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3 168 to our knowledge however, there is no evidence reported of GWAS in studies that
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5 169 evaluate suicidal behavior in psychiatric patients. Therefore, the outcomes of the
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7 170 current protocol could provide essential information. The use of this type of genetic
8
9 171 tools will allow us to identify associated SNPs, missense and insertions and indels
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11 172 in mental illnesses such as schizophrenia and bipolar disorder, as well as their
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14 173 possible participation as predictors of suicidal behavior in a Mexican population.
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19 175 In conclusion, these findings could give important information to improve the design
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21 176 of future chips for molecular diagnosis of psychiatric disorders in Mexicans, which
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23 177 will be very useful in the prevention, diagnosis and prognosis of suicidal behavior in
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25 178 Mexico. Moreover, the findings will give a better perspective of the genetic
26
27 179 background as a predictor of suicidal behavior in psychiatric diseases. Hence, the
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29 180 outcomes would be useful in genetic research as well as in prevention and early
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31 181 diagnosis of suicidal behavior in Mexicans
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3 183 **Ethics and disseminations:**
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5 184 The study will be conducted in compliance with local regulations and internationally
6
7 185 established principles of the Declaration of Helsinki (59th General Assembly, Seoul,
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9 186 Korea, October 2008). This study was approved by the ethics and investigation
10
11 187 committees of the National Institute of Genomic Medicine on July 22nd 2015, No.
12
13 188 CEI 215/13. Before inclusion, all patients are required to sign an informed consent
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15 189 form.
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21 191 **Consent for publication:**
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23 192 Not applicable.
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28 194 **Availability of data and material:**
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30 195 Available upon request from the corresponding author
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35 197 **Competing interest statement:**
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37 198 The authors declare to have no competing interests.
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41
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47
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50
51 204 **Author contributions:**
52

53 205 ADGM, HN and JJMM conceived the study, participated in its design, helped to draft
54
55 206 the manuscript and mentored TBGC. CATZ, ADGM and JJMM critically revised
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57
58
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3 207 successive drafts and provided important intellectual input. CATZ and TBGC
4
5 208 coordinated and supervised the integration of the manuscript. ES, IEJR, HN and
6
7 209 ADGM contributed to developing the analytic plan proposed for this study. All authors
8
9
10 210 read and approved the final manuscript.

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12 211

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16
17 213 The protocol study will be a doctoral thesis of TBGC.
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231 **References**

- 232 1. Perlis RH, Huang J, Purcell S, et al. Genome-wide association study of suicide attempts in mood
233 disorder patients. *The American journal of psychiatry* 2010;167(12):1499-507. doi:
234 10.1176/appi.ajp.2010.10040541 [published Online First: 2010/11/03]
- 235 2. Pulay AJ, Rethelyi JM. Multimarker analysis suggests the involvement of BDNF signaling and
236 microRNA biosynthesis in suicidal behavior. *American journal of medical genetics Part B,
237 Neuropsychiatric genetics : the official publication of the International Society of Psychiatric
238 Genetics* 2016;171(6):763-76. doi: 10.1002/ajmg.b.32433 [published Online First:
239 2016/02/28]
- 240 3. Mullins N, Perroud N, Uher R, et al. Genetic relationships between suicide attempts, suicidal
241 ideation and major psychiatric disorders: a genome-wide association and polygenic scoring
242 study. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official
243 publication of the International Society of Psychiatric Genetics* 2014;165B(5):428-37. doi:
244 10.1002/ajmg.b.32247 [published Online First: 2014/06/26]
- 245 4. Perroud N, Uher R, Ng MY, et al. Genome-wide association study of increasing suicidal ideation
246 during antidepressant treatment in the GENDEP project. *Pharmacogenomics J*
247 2012;12(1):68-77. doi: 10.1038/tpj.2010.70 [published Online First: 2010/09/30]
- 248 5. Stein MB, Ware EB, Mitchell C, et al. Genomewide association studies of suicide attempts in US
249 soldiers. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official
250 publication of the International Society of Psychiatric Genetics* 2017;174(8):786-97. doi:
251 10.1002/ajmg.b.32594 [published Online First: 2017/09/14]
- 252 6. Sokolowski M, Wasserman J, Wasserman D. Rare CNVs in Suicide Attempt include Schizophrenia-
253 Associated Loci and Neurodevelopmental Genes: A Pilot Genome-Wide and Family-Based
254 Study. *PloS one* 2016;11(12):e0168531. doi: 10.1371/journal.pone.0168531 [published
255 Online First: 2016/12/29]
- 256 7. Levine SZ, Goldberg Y, Yoffe R, et al. Suicide attempts in a national population of twins concordant
257 for psychoses. *European neuropsychopharmacology : the journal of the European College of
258 Neuropsychopharmacology* 2014;24(8):1203-9. doi: 10.1016/j.euroneuro.2014.05.014
259 [published Online First: 2014/06/28]
- 260 8. Linker J, Gillespie NA, Maes H, et al. Suicidal ideation, depression, and conduct disorder in a
261 sample of adolescent and young adult twins. *Suicide & life-threatening behavior*
262 2012;42(4):426-36. doi: 10.1111/j.1943-278X.2012.00101.x [published Online First:
263 2012/06/01]
- 264 9. Petersen L, Sorensen TI, Kragh Andersen P, et al. Genetic and familial environmental effects on
265 suicide attempts: a study of Danish adoptees and their biological and adoptive siblings.
266 *Journal of affective disorders* 2014;155:273-7. doi: 10.1016/j.jad.2013.11.012 [published
267 Online First: 2013/12/05]
- 268 10. Roy A, Rylander G, Sarchiapone M. Genetics of suicides. Family studies and molecular genetics.
269 *Annals of the New York Academy of Sciences* 1997;836:135-57. [published Online First:
270 1998/06/09]
- 271 11. Mirkovic B, Cohen D, Laurent C, et al. A case-control association study of 12 candidate genes and
272 attempted suicide in French adolescents. *International journal of adolescent medicine and
273 health* 2017 doi: 10.1515/ijamh-2017-0089 [published Online First: 2017/09/14]
- 274 12. Tombacz D, Maroti Z, Kalmar T, et al. High-Coverage Whole-Exome Sequencing Identifies
275 Candidate Genes for Suicide in Victims with Major Depressive Disorder. *Scientific reports*
276 2017;7(1):7106. doi: 10.1038/s41598-017-06522-3 [published Online First: 2017/08/05]

- 1
2
3 277 13. Gross JA, Bureau A, Croteau J, et al. A genome-wide copy number variant study of suicidal
4 278 behavior. *PloS one* 2015;10(5):e0128369. doi: 10.1371/journal.pone.0128369 [published
5 279 Online First: 2015/05/27]
6 280 14. Galfalvy H, Haghghi F, Hodgkinson C, et al. A genome-wide association study of suicidal
7 281 behavior. *American journal of medical genetics Part B, Neuropsychiatric genetics : the
8 282 official publication of the International Society of Psychiatric Genetics* 2015;168(7):557-63.
9 283 doi: 10.1002/ajmg.b.32330 [published Online First: 2015/06/17]
10 284 15. Galfalvy H, Zalsman G, Huang YY, et al. A pilot genome wide association and gene expression
11 285 array study of suicide with and without major depression. *The world journal of biological
12 286 psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry*
13 287 2013;14(8):574-82. doi: 10.3109/15622975.2011.597875 [published Online First:
14 288 2011/11/09]
15 289 16. Laje G, Allen AS, Akula N, et al. Genome-wide association study of suicidal ideation emerging
16 290 during citalopram treatment of depressed outpatients. *Pharmacogenet Genomics*
17 291 2009;19(9):666-74. doi: 10.1097/FPC.0b013e32832e4bcd [published Online First:
18 292 2009/09/03]
19 293 17. Zai CC, Goncalves VF, Tiwari AK, et al. A genome-wide association study of suicide severity scores
20 294 in bipolar disorder. *Journal of psychiatric research* 2015;65:23-9. doi:
21 295 10.1016/j.jpsychires.2014.11.002 [published Online First: 2015/04/29]
22 296 18. Menke A, Domschke K, Czamara D, et al. Genome-wide association study of antidepressant
23 297 treatment-emergent suicidal ideation. *Neuropsychopharmacology : official publication of
24 298 the American College of Neuropsychopharmacology* 2012;37(3):797-807. doi:
25 299 10.1038/npp.2011.257 [published Online First: 2011/10/28]
26 300 19. Bani-Fatemi A, Graff A, Zai C, et al. GWAS analysis of suicide attempt in schizophrenia: Main
27 301 genetic effect and interaction with early life trauma. *Neurosci Lett* 2016;622:102-6. doi:
28 302 10.1016/j.neulet.2016.04.043 [published Online First: 2016/04/26]
29 303 20. Schosser A, Butler AW, Ising M, et al. Genomewide association scan of suicidal thoughts and
30 304 behaviour in major depression. *PloS one* 2011;6(7):e20690. doi:
31 305 10.1371/journal.pone.0020690 [published Online First: 2011/07/14]
32 306 21. Willour VL, Seifuddin F, Mahon PB, et al. A genome-wide association study of attempted suicide.
33 307 *Molecular psychiatry* 2012;17(4):433-44. doi: 10.1038/mp.2011.4 [published Online First:
34 308 2011/03/23]
35 309 22. Gonzalez-Castro TB, Nicolini H, Lanzagorta N, et al. The role of brain-derived neurotrophic factor
36 310 (BDNF) Val66Met genetic polymorphism in bipolar disorder: a case-control study,
37 311 comorbidities, and meta-analysis of 16,786 subjects. *Bipolar disorders* 2015;17(1):27-38.
38 312 doi: 10.1111/bdi.12227 [published Online First: 2014/07/22]
39 313 23. Gonzalez-Castro TB, Tovilla-Zarate C, Juarez-Rojop I, et al. Association of the 5HTR2A gene with
40 314 suicidal behavior: case-control study and updated meta-analysis. *BMC psychiatry*
41 315 2013;13:25. doi: 10.1186/1471-244x-13-25 [published Online First: 2013/01/15]
42 316 24. Gonzalez-Castro TB, Tovilla-Zarate CA, Juarez-Rojop I, et al. Association of 5HTR1A gene variants
43 317 with suicidal behavior: case-control study and updated meta-analysis. *Journal of psychiatric
44 318 research* 2013;47(11):1665-72. doi: 10.1016/j.jpsychires.2013.04.011 [published Online
45 319 First: 2013/08/06]
46 320 25. Guo Y, He J, Zhao S, et al. Illumina human exome genotyping array clustering and quality control.
47 321 *Nature protocols* 2014;9(11):2643-62. doi: 10.1038/nprot.2014.174 [published Online First:
48 322 2014/10/17]
49 323 26. Borges G, Orozco R, Medina Mora ME. [Risk index for attempted suicide in Mexico]. *Salud publica
50 324 de Mexico* 2012;54(6):595-606. [published Online First: 2013/01/16]

- 1
2
3 325 27. Chang CC, Chow CC, Tellier LC, et al. Second-generation PLINK: rising to the challenge of larger
4 326 and richer datasets. *GigaScience* 2015;4:7. doi: 10.1186/s13742-015-0047-8 [published
5 327 Online First: 2015/02/28]
6 328 28. Loh PR, Danecek P, Palamara PF, et al. Reference-based phasing using the Haplotype Reference
7 329 Consortium panel. *Nat Genet* 2016;48(11):1443-48. doi: 10.1038/ng.3679 [published Online
8 330 First: 2016/10/28]
9 331 29. McCarthy S, Das S, Kretzschmar W, et al. A reference panel of 64,976 haplotypes for genotype
10 332 imputation. *Nat Genet* 2016;48(10):1279-83. doi: 10.1038/ng.3643 [published Online First:
11 333 2016/08/23]
12 334 30. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 2014;511(7510):421-
13 335 7. doi: 10.1038/nature13595 [published Online First: 2014/07/25]
14 336 31. Euesden J, Lewis CM, O'Reilly PF. PRSice: Polygenic Risk Score software. *Bioinformatics*
15 337 2015;31(9):1466-8. doi: 10.1093/bioinformatics/btu848 [published Online First:
16 338 2015/01/01]
17 339 32. Yang J, Lee SH, Goddard ME, et al. GCTA: a tool for genome-wide complex trait analysis. *Am J*
18 340 *Hum Genet* 2011;88(1):76-82. doi: 10.1016/j.ajhg.2010.11.011 [published Online First:
19 341 2010/12/21]
20 342 33. Bakshi A, Zhu Z, Vinkhuyzen AA, et al. Fast set-based association analysis using summary data
21 343 from GWAS identifies novel gene loci for human complex traits. *Sci Rep* 2016;6:32894. doi:
22 344 10.1038/srep32894 [published Online First: 2016/09/09]
23 345 34. Yang J, Lee SH, Goddard ME, et al. Genome-wide complex trait analysis (GCTA): methods, data
24 346 analyses, and interpretations. *Methods in molecular biology (Clifton, NJ)* 2013;1019:215-36.
25 347 doi: 10.1007/978-1-62703-447-0_9 [published Online First: 2013/06/13]
26 348 35. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations
27 349 using PolyPhen-2. *Current protocols in human genetics* 2013;Chapter 7:Unit7.20. doi:
28 350 10.1002/0471142905.hg0720s76 [published Online First: 2013/01/15]
29 351 36. Ng PC, Henikoff S. Predicting deleterious amino acid substitutions. *Genome research*
30 352 2001;11(5):863-74. doi: 10.1101/gr.176601 [published Online First: 2001/05/05]
31 353 37. McLaren W, Gil L, Hunt SE, et al. The Ensembl Variant Effect Predictor. *Genome biology*
32 354 2016;17(1):122. doi: 10.1186/s13059-016-0974-4 [published Online First: 2016/06/09]
33 355 38. An integrated encyclopedia of DNA elements in the human genome. *Nature* 2012;489(7414):57-
34 356 74. doi: 10.1038/nature11247 [published Online First: 2012/09/08]
35 357 39. Kircher M, Witten DM, Jain P, et al. A general framework for estimating the relative
36 358 pathogenicity of human genetic variants. 2014;46(3):310-5. doi: 10.1038/ng.2892
37 359 40. Romero-Pimentel AL, Mendoza-Morales RC, Fresan A, et al. Demographic and Clinical
38 360 Characteristics of Completed Suicides in Mexico City 2014-2015. *Front Psychiatry*
39 361 2018;9:402. doi: 10.3389/fpsy.2018.00402 [published Online First: 2018/09/25]
40 362 41. Bachmann S. Epidemiology of Suicide and the Psychiatric Perspective. *International journal of*
41 363 *environmental research and public health* 2018;15(7) doi: 10.3390/ijerph15071425
42 364 [published Online First: 2018/07/11]
43
44
45
46
47
48 365
49
50
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52
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54
55
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