PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Genome-Wide Association Study of suicide attempt in a Mexican population: A study protocol
AUTHORS	Genis-Mendoza, Alma; González-Castro, Thelma; Tovilla-Zárate, Carlos; Martínez Magaña, José Jaime; Juárez-Rojop, Isela; Sarmiento, Emmanuel; Nicolini, Humberto

VERSION 1 - REVIEW

REVIEWER	Peter Holmans MRC Centre for Neuropsychiatric Genetics and Genomics, Cardiff University School of Medicine, Hadyn Ellis Building, Cardiff CF24 4HQ, United Kingdom
REVIEW RETURNED	18-Jul-2018

GENERAL COMMENTS	This is an interesting study, the first GWAS of suicidal behaviour in Mexicans. The methodology is generally appropriate and clearly described. There should be some clarification of what association tests are to be performed. For example: schizophrenics with suicidal behaviour vs. schizophrenics without sucidal behaviour ? How do the healthy controls fit in ? They could be used to test for associations with schizophrenia/bipolar disorder but not suicidal behaviour itself (except in suicide attempters without psychiatric comorbidity) The sample size (N=700) is fairly low, particularly if schizophrenics and bipolars are treated separately. It would be useful to see a breakdown of expected numbers in the various case groups. It would also be interesting to see a power calculation based on effect sizes observed in previous suicide GWAS. Given the small sample, the applicants could try and increase power by utilising previous GWAS of suicide (via polygenic scores) - this could also be done for other psychiatric disorders (e.g. schizophrenia) for which very large, well-powered studies are available. The authors propose to impute on the 1000 genomes reference panel - they might wish to consider using the Haplotype Reference Consortion (HRC) panel, which includes the 1000 genomes data. Currently this mainly contains European-origin data, but there are plans to expand to other ethnicities

REVIEWER	Hanga Galfalvy Departments of Biostatistics and Psychiatry, Columbia University, USA Dr. Galfalvy's family owns equity worth about $5,000 in Illumina, Inc.
REVIEW RETURNED	18-Sep-2018

GENERAL COMMENTS	The manuscript “Genome-wide association study of suicide behavior in psychiatric disorders: A protocol in Mexican population” describes a planned study for 700 psychiatric patients and 500 healthy controls. The language of the article is very good, and the planned design includes several modern analytic techniques besides the “classical GWAS” (their term). As the authors note, there is not much published about the genetics of suicide in Mexican populations, and such a study could provide some valuable data for further research. However, the information presented in the current proposal is not sufficient to determine exactly what group comparisons will be tested, whether the adjustments made in the models will be adequate, or the expected effect sizes to be detected. Another round of review may be needed after more information is provided. Specific comments 1. The planned sample size for suicide attempters is not quoted either in the Abstract, or in the Sample description in the Methods section. Please include this information up front. 2. Due to the well-known heterogeneity of the Mexican population, cited in the limitations, a reader would expect to see an adjustment for ancestry in the models– yet there is no description in the Methods section on whether or how this will be performed. Is there any reason why the authors use the Genetic Relationship Matrix (GRM, which is useful to adjust for familial relationships that occur in genetic epidemiology) and do not use ancestry factors? Please include a reference that shows that GRM is sufficient adjustment in terms of the Mexican population, otherwise consider including an additional step in the analysis, a Multidimensional Scaling (MDS) type approach to deriving the ancestral factors, and adjusting for them in the model. 3. The first planned analysis is a “classical GWAS”. However, the description of the contrasts to be tested is missing. The sample section contains the following sentence: “Subsequently, the sample will be stratified as follows: schizophrenics with and without suicide attempt, bipolar with and without suicide attempt, only suicide attempters and finally, healthy subjects as controls.”. Does this mean that the authors plan to fit a 6-group comparison? Based on the limited sample size, that would results in extremely limited power. Please provide more information on the actual hypothesis tested, including all comparisons, and the planned adjustments for multiple testing. 4. Relatedly, some discussion of the expected statistical power for the GWAS would be appreciated, in terms of minimal detectable effect sizes and their comparison to those of previously identified biomarkers for suicidal behavior.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1 Peter Holmans

Institution and Country: MRC Centre for Neuropsychiatric Genetics and Genomics, Cardiff University School of Medicine, Hadyn Ellis Building, Cardiff CF24 4HQ, United Kingdom

Comment [1]: This is an interesting study, the first GWAS of suicidal behaviour in Mexicans.

Reply [1]: Thank you very much

Comment [2]: The methodology is generally appropriate and clearly described. There should be some clarification of what association tests are to be performed. For example: schizophrenics with suicidal behaviour vs. schizophrenics without sucidal behaviour? How do the healthy controls fit in ? They could be used to test for associations with schizophrenia/bipolar disorder but not suicidal behaviour itself (except in suicide attempters without psychiatric comorbidity)

Reply [2]: We are agree. We recognized the confusion of our metrological section due to in basis with your comment and the comment 4 of the reviewer 2 we detail more in “Sample population and setting” and “statistical analyses” of methods an analyses section.

See:

Methods and analysis, Sample population and setting: Page 5; lines 109-119

Methods and analysis, statistical analyses: Page 9; lines 197-202

Comment [3]: The sample size (N=700) is fairly low, particularly if schizophrenics and bipolars are treated separately. It would be useful to see a breakdown of expected numbers in the various case groups. It would also be interesting to see a power calculation based on effect sizes observed in previous suicide GWAS.

Reply [3]: We are agree. Due that based on your comment and the comment 5 of the reviewer 2 we calculated the power based on previously report [1].

See:

Methods and analysis, power analysis calculation: Page 9; Lines 204-208

Comment [4]: Given the small sample, the applicants could try and increase power by utilising previous GWAS of suicide (via polygenic scores) - this could also be done for other psychiatric disorders (e.g. schizophrenia) for which very large, well-powered studies are available.

Reply [4]: Thank you very much, we follows you suggestion and we added polygenic risk score calculation section in the methods

See:

Methods and analysis, polygenic risk scores calculation: Page 8; Lines 174-182

Comment [5]: The authors propose to impute on the 1000 genomes reference panel - they might wish to consider using the Haplotype Reference Consortion (HRC) panel, which includes the 1000 genomes data. Currently this mainly contains European-origin data, but there are plans to expand to other ethnicities

Reply [5]: Thanks, we will follow your suggestion

See: Methods and analysis, genotyping: Page 8; Lines 169-172

Reviewer 2: Hanga Galfalvy

Institution and Country: Departments of Biostatistics and Psychiatry, Columbia University, USA

Comment [1]: The manuscript “Genome-wide association study of suicide behavior in psychiatric disorders: A protocol in Mexican population” describes a planned study for 700 psychiatric patients and 500 healthy controls. The language of the article is very good, and the planned design includes several modern analytic techniques besides the “classical GWAS” (their term). As the authors note, there is not much published about the genetics of suicide in Mexican populations, and such a study could provide some valuable data for further research. However, the information presented in the current proposal is not sufficient to determine exactly what group comparisons will be tested, whether the adjustments made in the models will be adequate, or the expected effect sizes to be detected. Another round of review may be needed after more information is provided.

Reply [1]: We agree with your comments. In fact, based on yours and the comment 2 of the reviewer 1 we perform some modification.

See:

Methods and analysis, Sample population and setting: Page 5; lines 109-118

Methods and analysis, statistical analyses: Page 10; lines 197-202

Comment [2]: The planned sample size for suicide attempters is not quoted either in the Abstract, or in the Sample description in the Methods section. Please include this information up front.

Reply [2]: Thanks, We added the information required

See:

Abstract section, methods and analysis: Page 2; Lines 38-45

Methods and analysis, sample population and setting: Page 5, Lines 109-119

Comment [3]: Due to the well-known heterogeneity of the Mexican population, cited in the limitations, a reader would expect to see an adjustment for ancestry in the models– yet there is no description in the Methods section on whether or how this will be performed. Is there any reason why the authors use the Genetic Relationship Matrix (GRM, which is useful to adjust for familial relationships that occur in genetic epidemiology) and do not use ancestry factors? Please include a reference that shows that GRM is sufficient adjustment in terms of the Mexican population, otherwise consider including an additional step in the analysis, a Multidimensional Scaling (MDS) type approach to deriving the ancestral factors, and adjusting for them in the model.

Reply [3]: Thanks, we agree with your suggestion. We will used MDS approach

See:

Methods and analysis, genotyping: Page 7-8; Lines 164-169

Comment [4]: The first planned analysis is a “classical GWAS”. However, the description of the contrasts to be tested is missing. The sample section contains the following sentence: “Subsequently, the sample will be stratified as follows: schizophrenics with and without suicide attempt, bipolar with and without suicide attempt, only suicide attempters and finally, healthy subjects as controls.”. Does

this mean that the authors plan to fit a 6-group comparison? Based on the limited sample size, that would results in extremely limited power. Please provide more information on the actual hypothesis tested, including all comparisons, and the planned adjustments for multiple testing.

Reply [4]: We added the information required.

Introduction: Page 5; Lines 102-105

Methods and analysis, Sample population and setting: Page 5; lines 108-119

Methods and analysis, statistical analyses: Page 9; lines 197-202

Comment [5]: Relatedly, some discussion of the expected statistical power for the GWAS would be appreciated, in terms of minimal detectable effect sizes and their comparison to those of previously identified biomarkers for suicidal behavior.

Reply [5]: We are agree. Due that based on your comment and the comment 3 of the reviewer 1 we calculated the power based on previously report [1].

See:

Methods and analysis, power analysis calculation: Page 9; Lines 204-208

VERSION 2 – REVIEW

REVIEWER	Peter Holmans MRC Centre for Neuropsychiatric Genetics and Genomics, Cardiff University School of Medicine, Hadyn Ellis Building, Cardiff CF24 4HQ, United Kingdom
REVIEW RETURNED	06-Dec-2018

GENERAL COMMENTS	The manuscript is much clearer than it was before. I have some minor comments: 1. While the inclusion of a power study (lines 202-206) is good, the study could be more clearly described. For example: what effect size (odds ratio) is being tested ? What is the significance level ? ($p<0.05$?). What is P0, and why has a value of 0.08 been chosen ? The authors could pick the top hit from the Galfalvy et al. paper as the model to be tested (MAF=0.2, OR=1.8). Since the effect size is likely to be inflated due to winner's curse, they could also test the lower bound of the 95% confidence interval (OR=1.42) 2. The accepted p-value criterion for genome-wide significance in a GWAS is $p<5x10-8$. So, there's no need to do a Bonferroni correction to determine significance (lines 198-200). Power calculations (see above) should be performed at a significance level of 5x10-8.

REVIEWER	Hanga Galfalvy Departments of Psychiatry, Columbia University Vagelos College of Physicians and Surgeons and Department of Biostatistics, Columbia University Mailman School of Public Health

REVIEW RETURNED	28-Nov-2018

GENERAL COMMENTS	The authors have satisfactorily addressed all my comments, however they forgot to include the expected effect size, in the form of an odds ratio, in the Power Analysis section MAF, P0 and effect size are all required to calculate power in Quanto for any given sample size, but only two of the three were provided. A reference to a prior study for why this effect size is realistically expected to occur would also be good to include, especially if the effect size value used was more than 2. This oversight should be corrected. Further review afterwards should be at the editor's discretion.

VERSION 2 – AUTHOR RESPONSE

Reviewer: Hanga Galfalvy

Comment: The authors have satisfactorily addressed all my comments, however they forgot to include the expected effect size, in the form of an odds ratio, in the Power Analysis section MAF, P0 and effect size are all required to calculate power in Quanto for any given sample size, but only two of the three were provided. A reference to a prior study for why this effect size is realistically expected to occur would also be good to include, especially if the effect size value used was more than 2. This oversight should be corrected. Further review afterwards should be at the editor's discretion.

Reply: Thank you very much. We try to added the information required. (Methods section, statistical analysis: Page 9; Lines 133-142)

In this section we modified:

“Power analysis calculation

For the primary analysis of the GWAS suicide attempt using 700 cases and 500 controls, we will use a log additive model of inheritance, MAF of 25%, P0 of 0.08 the power will be 0.99. All the calculations will be performed using QUANTO 1.2.4. (http://biostats.usc.edu/software), as previously reported.”

To this:

Power analysis calculation

“For GWAS analysis, we performed power calculations in QUANTO 1.2.4 (http://biostats.usc.edu/software) 14. The analysis use a log additive model of inheritance to detect a power of 0.98 at significance threshold 5x10-08 to detect an effect size of OR ≥ 2 with a MAF of 0.25 and a P0 of 0.08. Also testing the lower bounds of the effect sizes of the variants, we observe a power of 0.82 with an effect size of 1.8, P0=0.08 and MAF=0.25 at the same significance level. The P0 that we used is the baseline risk of suicide attempt in our population based on previous reports 40 41. Therefore, our study will be powered to detect genetics effects.”

Reviewer: Peter Holmans

Comment [1]: While the inclusion of a power study (lines 202-206) is good, the study could be more clearly described. For example: what effect size (odds ratio) is being tested? What is the significance level ? ($p<0.05$?). What is P0, and why has a value of 0.08 been chosen ? The authors could pick the

top hit from the Galfalvy et al. paper as the model to be tested (MAF=0.2, OR=1.8). Since the effect size is likely to be inflated due to winner's curse, they could also test the lower bound of the 95% confidence interval (OR=1.42).

Reply [1]: Thanks, we try to follow your suggestion (Methods section, statistical analysis: Page 9; Lines 133-142). Furthermore we added the genome-wide significance (Methods section, statistical analysis: Page 9; Line 131).

In this section we modified:

“Power analysis calculation

For the primary analysis of the GWAS suicide attempt using 700 cases and 500 controls, we will use a log additive model of inheritance, MAF of 25%, P0 of 0.08 the power will be 0.99. All the calculations will be performed using QUANTO 1.2.4. (http://biostats.usc.edu/software), as previously reported.”

To this:

Power analysis calculation

“For GWAS analysis, we performed power calculations in Quanto 1.2.4 (http://biostats.usc.edu/software) 14. The analysis use a log additive model of inheritance to detect a power of 0.98 at significance threshold 5x10-08 to detect an effect size of OR ≥ 2 with a MAF of 0.25 and a P0 of 0.08. Also testing the lower bounds of the effect sizes of the variants, we observe a power of 0.82 with an effect size of 1.8, P0=0.08 and MAF=0.25 at the same significance level. The P0 that we used is the baseline risk of suicide attempt in our population based on previous reports 40 41. Therefore, our study will be powered to detect genetics effects.”

Comment [2]: The accepted p-value criterion for genome-wide significance in a GWAS is p<5x10-8. So, there's no need to do a Bonferroni correction to determine significance (lines 198-200). Power calculations (see above) should be performed at a significance level of 5x10-8.

Reply [2]: We are agree, we excluded the Bonferroni correction. We set the significance in (Methods section, statistical analysis: Page 9; Line 131) and the power calculation was performed at the same significance level.

In this section we eliminated:

For the joint analysis, we will investigate the possibility of a new association within the genome; therefore, multiple testing corrections will be conducted using the Bonferroni correction and permutation; the corrected p value will depend on the total number of independent tests.

And instead we included:

“Genome wide significance will be set at P <5x10-08”

VERSION 3 – REVIEW

REVIEWER	Peter Holmans Cardiff University, United Kingdom
REVIEW RETURNED	21-Dec-2018

GENERAL COMMENTS	The authors have done a reasonable job with their power calculation. The only change I suggest they make is to remove the power for OR=2 and say that the OR of 1.8 was taken from a prior study (Galflavy et al).

REVIEWER	Hanga Galfalvy Departments of Psychiatry and Biostatistics, Columbia University
REVIEW RETURNED	07-Jan-2019

GENERAL COMMENTS	No further comments, the authors addressed all of my concerns.

VERSION 3 – AUTHOR RESPONSE

Reviewer: Peter Holmans

Comment: The authors have done a reasonable job with their power calculation. The only change I suggest they make is to remove the power for OR=2 and say that the OR of 1.8 was taken from a prior study (Galflavy et al).

Reply: Thank you for your suggestion. We remove the OR=2, see Methods and analysis; Power analysis calculation: Page 9; Lines 133-140

Reviewer: Hanga Galfalvy

Comment: No further comments, the authors addressed all of my concerns.

Reply: Thank you very much.