PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Multi-Gene Interactions and the Prediction of Depression in the Wisconsin Longitudinal Study
AUTHORS	Nicholas S. Roetker, James A. Yonker, Chee Lee, Vicky Chang, Jacob Basson, Carol L. Roan, Taissa S. Hauser, Robert M. Hauser and Craig S. Atwood

VERSION 1 - REVIEW

REVIEWER	Xudong Huang, Ph.D. Lab Co-Director and Assistant Prof. Massachusetts General Hospital and Harvard Medical School Boston, MA 02114, USA
REVIEW RETURNED	13/02/2012

GENERAL COMMENTS	In manuscript by Roetker et al., a classification tree approach-recursive partitioning (RP) has been validated as a powerful and efficient technique to exam gene-gene interactions and identify SNP associations predictive of depression. The experimental methodology is solid and well described. The experimental evidence is convincing and the finding is intriguing. It demonstrates that this exploratory analysis techniques can be utilized as a general method to reveal genetic and molecular pathway interactions associated with human disease etiopathology. The manuscript was also well written. Thus, it warrants a publication in BMJ Open. However, the manuscript can be improved if the following minor revision is made: Critique: a) Almost of all human subjects in this study are non-Hispanic white. This could have a conflict with NIH Guidelines on the Inclusion of Women and Minorities as Subjects as the study are supported by NIH/NIA. A few sentences of justifying or clarifying human subject enrollment in the manuscript may remove any concern about this issue.

REVIEWER	Dr Alessandra Minelli Department of Biomedical Sciences and Biotechnologies - Biology and Genetic Division - University of Brescia. Viale Europa 11, 25123 Brescia, Italy I have no competing interests
REVIEW RETURNED	16/04/2012

GENERAL COMMENTS	Manuscript ID bmjopen-2012-000944, entitled "Multi-Gene Interactions and the Prediction of Depression in the Wisconsin

	Longitudinal Study." The study is focused on gene x gene interactions in depression using the RP tree approach. Major revision 1- The main goal of the study is not clear. Please, improve the introduction and discussion, clarifying how your new findings on GxG interaction are relevant in genetic of depression studies and better demonstrate the utility of RP analysis. 2 - In the “study participants” is mentioned a sample of 10317. In the genotyping section is mentioned a sample of 7101 subjects. The final sample genotyped is made of 4792 subjects which 711 with depression. Please 1) describe better the socio demographic features of the final sample. 2) Explain better why the RP classification tree analysis was performed on the whole sample of subjects and not only depressed patients. 3) specify the percentage and numbers of cases and controls and not the incidence of depression. 4) specify criteria used to define the group of controls (Have these subjects received a screening for the exclusion of DSM-IV Axis I disorders?) 3) Please clarify also in the results section and in the table 1, that the rs1800497 of the gene ANKK1, is historically known as the DRD2 Taq1A because it is confounding for the reader. 4) Figures 1 and 2: In every steps some subjects are lost. Please clarify why, and indicate in the figures the percentage and the number of cases and controls. 5) Even if you indicated that the characteristics of WLS cohort may be found elsewhere, please describe principal inclusion/exclusioncriteria. Minor Revision: Introduction: Age is one of many factors that influences depression. Describe shortly others factors. Methods: To indicate the questions for the diagnosis of depression is not relevant. Please indicate diagnosis of DSM-IV MDD.

VERSION 1 – AUTHOR RESPONSE

Reviewer: Xudong Huang, Ph.D.

a) Almost of all human subjects in this study are non-Hispanic white. This could have a conflict with NIH Guidelines on the Inclusion of Women and Minorities as Subjects as the study are supported by NIH/NIA. A few sentences of justifying or clarifying human subject enrollment in the manuscript may remove any concern about this issue.

Our revision more fully describes the ancestral background of our study population. See METHODS (Study Participant and Surveys): “Data were collected from the WLS, a random sample originally comprised of 10, 317 men and women who graduated from Wisconsin high schools in 1957. Later in 1977, the WLS began interviewing one randomly selected sibling of each graduate, when possible. The cohort reflects the ancestral makeup of the late-1950s Wisconsin population in that participants are almost entirely non-Hispanic white males and females. In general, the sample is broadly representative of older white Americans with at least a high school education.”

Reviewer: Dr Alessandra Minelli

1- The main goal of the study is not clear. Please, improve the introduction and discussion, clarifying how your new findings on GxG interaction are relevant in genetic of depression studies and better demonstrate the utility of RP analysis.
We have clarified the goals of the study. “The goals of this study were therefore to 1) explore G x G interactions that might better predict the genetic factors involved in the etiology of depression, and 2) to determine the utility of machine learning algorithms (recursive partitioning) to identify genetic interactions.”
We feel that we adequately describe how RP can be used for genetic studies in the discussion under the section “The Utility of Recursive Partitioning and Logistic Regression for Identification of Gene-Gene Interactions”.

2 - In the “study participants” is mentioned a sample of 10317. In the genotyping section is mentioned a sample of 7101 subjects. The final sample genotyped is made of 4792 subjects which 711 with depression. Please
1) describe better the socio demographic features of the final sample.
We now include age, marital status, educational attainment, and household income for our final sample. See METHODS (Statistical Analysis): “Analyses were limited to the 4,811 pooled graduates and siblings for whom we have depression and genotype information (Note: individuals with more than 10% missing genotype data were not included). The average age among this sample was just under 65 years in 2004. 80% were married, and the average amount of post-high school educational attainment was 2 years. Median household income in 1993 was $56,700.”

2) Explain better why the RP classification tree analysis was performed on the whole sample of subjects and not only depressed patients.
RP classification trees require a binary outcome variable (diseased vs. non diseased), as splits in the trees seek to separate diseased individuals from non-diseased individuals. Thus, in this study we have looked at depressed vs. non-depressed subjects. We have clarified this in our revision. See METHODS (Statistical Analysis: Recursive Partitioning (RP)): “RP is a data mining tool for revealing trends that relate a dependent variable (depressed vs. non-depressed) to various predictor variables (SNPs).”

3) specify the percentage and numbers of cases and controls and not the incidence of depression.
We have changed Figures 1 and 2 to display percentage depressed and number of controls/cases.

4) specify criteria used to define the group of controls (Have these subjects received a screening for the exclusion of DSM-IV Axis I disorders?)
We have clarified this point in the manuscript. See METHODS (Study Participants and Surveys): “Symptom questions asked whether the two week period was accompanied with a) any weight loss, b) trouble sleeping, c) feeling tired, d) feeling bad upon waking, e) losing interest, f) trouble concentrating, or g) thoughts about death. Those answering YES to 3 or more of these symptom questions were classified as having depression (15). Those answering YES to 2 or fewer symptom questions and all those answering NO to the initial stem question were classified as controls.”
Subjects were not excluded based on DSM-IV Axis I disorders.

3) Please clarify also in the results section and in the table 1, that the rs1800497 of the gene ANKK1, is historically known as the DRD2 Taq1A because it is confounding for the reader.
In our revision we have noted that ANKK1 rs1800497 is also known as the DRD2 Taq1A in Table 1, Figure 1, and the abstract and results.

4) Figures 1 and 2: In every steps some subjects are lost. Please clarify why, and indicate in the figures the percentage and the number of cases and controls.
Our revision clarifies parameter settings used in rpart. The usesurrogate parameter was set to 0 so

that subjects missing in the primary split variable do not progress further down the tree. Thus, the reason why subjects are lost in every step is due to various missing genotype information among subjects (i.e., a particular subject might be included in the primary split, but in a subsequent split we might not have his or her genotype for that particular SNP, so thereafter they drop out of that branch). Based on this, we lose approximately 1.5% of data per split in men and 1.4% of data per split in women. We have clarified in the manuscript the specific persons considered in the final sample: "Analyses were limited to the 4,792 pooled graduates and siblings for whom we have depression and genotype information (Note: individuals with more than 10% missing genotype data were excluded from the dataset)." Note that among these 4,792 individuals, genotyping efficiency of the 78 SNPs was very high, averaging only approximately 1 missing SNP genotype per person.

5) Even if you indicated that the characteristics of WLS cohort may be found elsewhere, please describe principal inclusion/exclusion criteria.
Please see response to Reviewer 1, and Reviewer 2, Point 2 (1) above

Minor Revision:
Introduction: Age is one of many factors that influences depression. Describe shortly others factors.
We describe briefly other factors influencing depression: "While many environmental factors—such as socioeconomic status, childhood abuse, and major life events—have important ties with depression, so too does gender and many genetic and epigenetic factors, making the disorder heterogeneous in nature (2)".

Methods: To indicate the questions for the diagnosis of depression is not relevant. Please indicate diagnosis of DSM-IV MDD.
The study is based on self-reported survey data, so our measures of depression are reliant on the CIDI-SF scale included in participant surveys. Although the DSM-IV MDD criteria are very similar to the CIDI-SF criteria, they are not identical.

Please note that the latest manuscript includes 19 additional participants who we unintentionally excluded previously. Although N-sizes changed slightly throughout the paper to reflect this, the significance of the results remain unchanged.

Please do not hesitate to contact me if you have any questions. I look forward to receiving your response.

Sincerely yours,

Craig S. Atwood, Ph.D.
Associate Professor, Department of Medicine
UW School of Medicine and Public Health
Research Director, Wisconsin Alzheimer's Institute

VERSION 2 – REVIEW

REVIEWER	Xudong Huang Associate Research Scientist and Assistant Prof. MGH and Harvard Medical School USA
REVIEW RETURNED	01/05/2012

GENERAL COMMENTS	The authors have addressed reviewers' comments and critiques nicely. The manucript is now in even better shape. In addition, the

	experimental design is sound, and the experimental methodology is solid and well described. The experimental results are convincing and the manuscript is well written. The study thus warrants a publication in BMJ Open. To this reviewer, no further revision is needed.

REVIEWER	Dr Alessandra Minelli Department of Biomedical Sciences and Biotechnologies - Biology and Genetic Division - University of Brescia. Viale Europa 11, 25123 Brescia, Italy I have no competing interests
REVIEW RETURNED	08/05/2012

GENERAL COMMENTS	Manuscript ID bmjopen-2012-000944, entitled "Multi-Gene Interactions and the Prediction of Depression in the Wisconsin Longitudinal Study." The study is focused on gene x gene interactions in depression using the RP tree approach. Not all the previous revision point were fully elucidated. Major revision 1- The introduction is still insufficient, clarifying how GxG interaction are relevant in genetic of depression studies and better demonstrate the utility of RP analysis. The introduction doesn't help the reader to understand this method, regarding its validity, if it's already been used in others studies and so on. 2 – Please clarify in the manuscript also this your answer "Our revision clarifies parameter settings used in rpart. The usesurrogate parameter was set to 0 so that subjects missing in the primary split variable do not progress further down the tree. Thus, the reason why subjects are lost in every step is due to various missing genotype information among subjects (i.e., a particular subject might be included in the primary split, but in a subsequent split we might not have his or her genotype for that particular SNP, so thereafter they drop out of that branch). Based on this, we lose approximately 1.5% of data per split in men and 1.4% of data per split in women." 3- You indicated the description of the sample not the inclusion criteria: please describe principal inclusion/exclusion criteria.

VERSION 2 – AUTHOR RESPONSE

Reviewer: Alessandra Minelli

The study is focused on gene x gene interactions in depression using the RP tree approach. Not all the previous revision point were fully elucidated.

Major revision
1- The introduction is still insufficient, clarifying how GxG interaction are relevant in genetic of depression studies and better demonstrate the utility of RP analysis. The introduction doesn't help the reader to understand this method, regarding its validity, if it's already been used in others studies and so on.

We have added the following sentence to the introduction to clarify that this method has recently been used to examine gene interactions in depression:

“The machine learning tool recursive partitioning has recently been used by Wong (13) to assess complex gene-gene interactions in depression. Wong notes that recursive partitioning is useful in that it quickly explores high dimensional data for non-linear effects that are non-biased and easily interpretable.”

2 – Please clarify in the manuscript also this your answer “Our revision clarifies parameter settings used in rpart. The usesurrogate parameter was set to 0 so that subjects missing in the primary split variable do not progress further down the tree. Thus, the reason why subjects are lost in every step is due to various missing genotype information among subjects (i.e., a particular subject might be included in the primary split, but in a subsequent split we might not have his or her genotype for that particular SNP, so thereafter they drop out of that branch). Based on this, we lose approximately 1.5% of data per split in men and 1.4% of data per split in women.”

The information in the above paragraph is noted in the Methods and Figure Legends.

3- You indicated the description of the sample not the inclusion criteria: please describe principal inclusion/exclusion criteria.

As noted, the WLS is “a random sample originally comprised of 10, 317 men and women who graduated from Wisconsin high schools in 1957”. Please see Sewell WH. As We Age : The Wisconsin Longitudinal Study, 1957-2001: Center for Demography and Ecology University of Wisconsin--Madison; 2001, for an indepth discussion of this cohort that has been used in over 300 publications.

We also have clarified the inclusion and exclusion criteria for depression in the methods section:

“Inclusion criteria for depression included any member of the WLS cohort who was depressed according to the Composite International Diagnostic Interview short-form (CIDI-SF). Individuals who answered YES to the question “Have you ever had a time in life lasting two weeks or more when nearly every day you felt sad, blue, depressed, or when you lost interest in most things like work, hobbies, or things you usually liked to do for fun?” and whose depression was not caused by alcohol, drugs, medications, or physical illness were asked further depression symptom questions. Symptom questions asked whether the two week period was accompanied with a) any weight loss, b) trouble sleeping, c) feeling tired, d) feeling bad upon waking, e) losing interest, f) trouble concentrating, or g) thoughts about death. Those answering YES to 3 or more of these symptom questions were classified as having depression (16). Those answering YES to 2 or fewer symptom questions and all those answering NO to the initial stem question were classified as controls.”

VERSION 2 – REVIEW

REVIEWER	Dr Alessandra Minelli Department of Biomedical Sciences and Biotechnologies - Biology and Genetic Division - University of Brescia. Viale Europa 11, 25123 Brescia, Italy I have no competing interests

REVIEW RETURNED	08/05/2012

The reviewer completed the checklist but made no further comments.