Author's response to reviews

Title: Association of the MAOA Promoter uVNTR Polymorphism with Suicide Attempts in Patients with Major Depressive Disorder

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Dear Editors of *BMC Medical Genetics,*

The authors of “Association of the MAOA Promoter uVNTR Polymorphism with Suicide Attempts in Patients with Major Depressive Disorder”, all appreciate your comments and learn much from you. We also appreciate you providing us opportunities to discuss and share some points with you.

We have also made some corrections according to the reviewers’ opinions. Based on the reviewer’s suggestions, we re-analyzed our data, and the results should that MAOA 4R allele is associated with enhanced vulnerability to suicide in depressed males, but not the community subjects. The MAOA 4R allele affects vulnerability to suicide through the mediating factor of depressive symptoms. These major findings may explain the inconsistency of past results in regards to the association of MAOA with depression and/or suicidal behaviors, using experimental case-control paradigm. Our study has attempted to enhance the inaccurate diagnostic grouping, and not adjusting for possible confounding factors in previous studies. The revised manuscript has also modified the English grammar by an English Editing company-Genedits. We sincerely hope the modified manuscript submitted will show a significant improvement.
Reviewer 1

1. Is there any evidence in terms of transcriptional activity showing that the 2-repeat (R) allele and the 5 R allele should be grouped together with the 3 R allele and the 4 R allele, respectively in the analysis? If there is no such evidence, the authors should exclude individuals who carried either the 2R or 5R allele from the analysis because they were very rare. (Major Compulsory Revision)

Answer: In our original manuscript we categorized the MAOA uVNTR alleles based on the findings of Sabol et al.\(^1\) and Deckert et al.’s\(^2\) studies which demonstrated the 3.5 and 4R allele may be associated with a higher MAOA activity compared to the 2R, 3R and 5R allele using luciferase reporter system. Therefore, we used the cut-off values of 3.5R allele to subdivide the observed MAOA alleles into dichotomous groups of long-form allele (high activity allele) versus short-form allele (low activity allele). Our previous study based on this grouping method also identified short-form allele was associated with good therapeutic outcome in patients with MDD who received mirtazapine.\(^3\) Given that 2R and 5R allele were rare in this studied cohort, and the biological functions of 2R and 5R was uncertain due to they were reported for a higher transactivating ability than 3R and 4R in two human cell lines (IMR-32 and Hela).\(^4\) We excluded the 2R and 5R alleles, as suggested by the reviewer, and only compared the frequencies of 3R and 4R allele, as well as homozygous and/or hemizygote for 3R and 4R. Results showed the 4R allele was statistically significantly higher in the MDD males than the male community controls ($\chi^2 = 4.182, p = 0.041$). Likewise, the 4R allele was more frequent in male MDD suicide attempters than the male community controls ($p = 0.170$) with a sample size of 22 males carrying 4R allele in the MDD suicide group ($n = 22$). This may indicate an association between 4R allele and depression, suicide or both. (See Result section, page 10-13)

2. In table 1, allele frequencies of female and total subjects should also be described. (Minor Essential Revision)

Answer: According to the reviewer’s request, the allele frequencies of female and total subjects of each group have been indicated and modifications changed in Table 1. (Page 28-29)

3. In table 1, numbers of recruited subjects (N) were different from the numbers of genotyped subjects (n). I feel that the authors should describe only individuals
with genotype from the Sample collection part on page 6 to avoid confusion. (Minor Essential Revision)

Answer: The numbers of the recruited subjects (N) have been removed and only those who have successfully genotyped in each group were shown in Table 1, according to the reviewer’s suggestion. (Page 28-29)

4. A possibly major problem of the statistical analysis is the dichotomization of genotypes (L7-9P10), i.e., the authors defined the “high activity group” as male carriers of the long form variants and female homozygotes for the long-form variants and the low activity as their counterparts. I feel skeptical about the validity of such dichotomization. Is there any scientific rationale and evidence for such dichotomization? The authors analyzed the data based on the dichotomization and obtained peculiar results; only female community suicide attempters showed smaller frequency (62.3%) of carriers of short-form alleles, yielding “significant” differences between the group and the other groups. Consequently, the main results were such that the MAOA long-form variant was associated with increased vulnerability to suicide in the general population, whilst in MDD subjects, suicidal behavior was associated with the MAOA short-form variant. I think such conclusions are incoherent and invalid as well because they were derived from the genotype data that may have happened to be unusually low in the female suicide attempters from the community (N=only 38; the smallest group). In modern behavioral genetics, one should not draw any conclusion from such a small sample size. (Major Compulsory Revision)

Answer: According to the reviewer’s suggestion, we compared the MAOA genotype distribution based on new dichotomization in previous studies. As a result, only the 4R hemizygote was significantly higher in the MDD males compared to that of male community controls and yield a borderline significance ($\chi^2 = 4.182, p = 0.041$). Likewise, the 4R allele was more frequent in male MDD suicide attempters than in male community controls, even though it did not reach a statistically significant level ($p = 0.170$) and the sample size of 4R allele male carriers was small in MDD suicide group ($n = 22$). This result may indicate that 4R allele was associated with depression, suicide or both. Structural equation modeling (SEM) was conducted to clarify the association between depression and suicide, as well as the interacting paths between the associated factors. According to the results from SEM analysis, with potential
confounding factors controlled, the 4R allele of MAOA uVNTR (0 referred to 4R, 1 referred to 3R) did not have a direct effect on suicide attempt in MDD patients through the mediating factor of depressive symptoms (MAOA-depression: $\beta = -0.12$, $p = 0.031$; depression-suicide: $\beta = 0.32$, $p < 0.001$). However, MAOA 4R allele was not found to increase the vulnerability of suicide in community cohorts. Thus MAOA 4R allele is associated with enhanced vulnerability to suicide in depressed males, but not community subjects. The MAOA 4R allele affects the vulnerability of suicide through the mediating of depressive symptoms. Nevertheless, further large-scale studies are still needed to verify the psychopathology of the relationships among MAOA uVNTR polymorphism, symptom profiles, and suicidal behavior. (See Result section)

5. According to my calculation, there seems to be a significant difference between female community suicide group and female MDD group ($p=0.002$ instead of $p=0.854$) (Table 2). (Major Compulsory Revision)

Answer: After the analyzing method of MAOA genotype was corrected based on the aforementioned manner, the inter-group difference between these two groups was not significant ($\chi^2 = 0.021$, $p = 0.885$). (Page 28-29)

6. Concerning MAOA gene on the X chromosome, the gene dose is different between males and females. And there are several sex differences in clinical depression and suicide behavior. Therefore, to be robust, the authors should analyze the data men and women separately. If the authors do so, there appear clear and simple results; the R4 allele was significantly more common in MDD and suicide attempters in men while there was no such association in women. This might be the main conclusion of the study. Since the R4 allele is associated with increased transcriptional activity, this result is biologically reasonable. (Major Compulsory Revision)

Answer: To control for the effect of gender, we have re-analyzed and presented the MAOA allele frequencies of men and women separately. Additionally, the factor of sex was controlled in the logistic regression and SEM analysis. The results of our study showed that MAOA 4R allele and/or 4R hemizygote were associated with increased vulnerability to depression among the male subjects in our studied cohorts ($p = 0.041$). Although this association was unseen in the logistic regression analysis,
however SEM showed an indirect effect of suicide in MDD through the mediating factor of depressive symptoms. Additionally, other than the length of the MAOA uVNTR polymorphism, other factors which were found to be associated with suicide attempt in MDD included the demographics of level of education, age, marital status, personality characteristics of extraversion, neuroticism and anxiety symptoms. On the other hand, within the suicide attempters, age and somatic symptoms were associated with depression. (See Result section)

7. It is unlikely that individuals who had less anxiety are at risk for suicide attempt, suggesting that the model used in the study was something unfit with the reality. (Major Compulsory Revision)

Answer: As pointed out by the reviewer, previous studies have found an association between anxiety and suicide. However, other studies have also found otherwise. Fawcett et al. found that although anxiety was associated with suicide within the first year, but not with 2-10 years. Thus, different subtypes of MDD may be related to suicide. For instance, Grunebaum et al. found the self-blame factor was related to suicide, but not anxiety measured using Hamilton Depression Rating Scales. One of the limitations of this study is that we used the CHQ, which is only a general measure of psychiatric symptoms. Thus we were unable to identify the different subtypes of anxiety which may have different effect on suicide behavior. (See page 18-19)

References
5. Zhang H, Smith GN, Liu X, Holden JJ. Association of MAOA, 5-HTT, and


Reviewer 2

1. Include the age and gender distribution in a table with demographics.

Answer: The age and gender distribution has been added into Table 1 according to reviewer’s suggestion. (See page 28-29)

2. The table 1 and 2 is confusing the analysis has been done considering the genotype in female better consider the allele as high and low. Refer to the other publication.

Answer: A paired-comparison regarding the frequencies of 3R and 4R allele in the studied groups was carried out. Results showed the 4R allele was significantly higher in MDD males compared to that of male community controls ($\chi^2 = 4.182, p = 0.041$). We subdivided the allelic variants further into dichotomous groups according previous studies,$^5,6$ which included female homozygote for the 4R or male hemizygote of 4R (high activity group) and carriers of the 3R allele (low activity group). The genotype distributions of homozygous and/or hemizygous for 3R vs. homozygous and/or hemizygous for 4R in the four groups studied are shown in Table 2. (See page 30)
3. The logistic regression table is confusing what is the main outcome?
Answer: Logistic regression was used to investigate within patients with MDD to
determine which factors increased the risk of suicide by comparing the two groups of
participants with MDD, one with suicide attempt and one without. Results showed
those younger in age, more neurotic or smoked increased the risk of suicide ($\beta = -0.04,$
$p = 0.002$; $\beta = 0.15, p = 0.017$; $\beta = 0.79, p = 0.031$), as shown in Table 3a. Parental
attachment, personality characteristics, mental health condition, demographics and
allelic distribution differences between the two groups with suicide attempt, one with
MDD and one without was also analyzed. Results showed, of the two groups with
suicide attempt, those younger in age, with more paternal care, and more somatic
symptoms were more likely to be in the MDD group ($\beta = -0.11, p < 0.001$; $\beta = 0.15, p$
$= 0.026; \beta = 1.11, p < 0.001$), as shown in Table 3b. Thus in logistic regression, no
association was found between MAOA allele and depression or suicide. This can be
due to the possibility that MAOA does not play a vital role in the effect of
vulnerability to suicide, depression, or both; or MAOA may have an indirect affect on
depression or suicide via a gene-gene, gene-to-non-genetic factor interacting manners.
Therefore, SEM was conducted to clarify the possible interacting paths between the
associated factors of suicide. (See Result section and Page 31-33)

4. 95% CI should be included in the logistic regression.
Answer: The values of 95% C.I. have been added into the results of genotype
distribution and logistic regression, in Tables 2 and 3. (See page 31-33)

5. The fact that 2 and 5 alleles are more common in the Asian population should be
discussed in the paper.
Answer: As suggested by the reviewer, the fact that 2R and 5R alleles are more
common in the Asian population has been discussed. The 2R and 5R allele are rare
MAOA allele in our populations, but 2R allele seems to be higher than that in
Japanese and Caucasians (0.2~1.3% vs. none), whereas 5 allele is not higher than
that of Caucasians (0.2~0.76% vs. 1.6%). Interestingly, in this study, 2R allele was
detected in all groups except community controls, whereas 5R allele was restrictedly
observed in the community control group. The experimental evidence from a cell
based assay study, which contradict to results from Sabol et al. and Deckert et al.,

1,2
has shown that the 2R and 5R alleles had a higher transactivity in triggering luciferase activity in two human cell lines (IMR-32 and Hela). Therefore these two MAOA alleles may have their role involved in biological functionality of MAOA enzyme and perhaps are disease-associated even though their frequencies are rare. (See page 14)

6. The smoking and the personality and age is not included in the regression this is a gap it would be important to compare logistic regression and structural equation modeling.

Answer: Parental attachment, personality characteristics, mental health condition, demographics and allelic distribution were analyzed in both the logistic regression and SEM analysis comparing two groups of patients with MDD, one with suicide attempt and one without, to determine the associated factors which increased the risk of suicide. The full model of logistic regression showed, those younger in age, more neurotic or smoked, increased the risk of suicide ($\beta = -0.04$, $p = 0.002$; $\beta = 0.15$, $p = 0.017$; $\beta = 0.79$, $p = 0.031$). Parental attachment, personality characteristics, mental health condition, demographics and allelic distribution differences between the two groups with suicide attempt, one with MDD and one without was also analyzed. Results showed, of the two groups with suicide attempt, those younger in age, with more paternal care, and more somatic symptoms were more likely to be in the MDD group. Compared the result from logistic regression and SEM, age and personality characteristics have a direct effect on depressed suicide, but smoking only correlated to depressed suicide in logistic regression ($p = 0.031$). This finding indicated which the possible confounding factors controlled; the effect of smoking on suicide becomes not statistically significant. We have modified this in our text from the reanalyzed results. (See page 31-34)

7. The structural equation modeling should include the age.

Answer: The factor of age has been added in our SEM analysis. Comparing the two groups of depressed subject, one with and the other without suicide attempt, results showed younger age was associated with depressed suicide ($\beta = -0.31$, $p < 0.001$). Moreover, the parsimonious SEM model comparing two groups with suicide, one with MDD and the other without, results also showed younger subjects who have attempted suicide were more likely to have MDD ($\beta = -0.41$, $p < 0.001$). (See page 34-35)
8. In the modeling there is anxiety as factor but no mention about how was measured.
Answer: The anxiety and depression were measured using the Chinese Health Questionnaire (CHQ). The CHQ is a 12-item screening instrument that is used to identify minor psychiatric disorders in individuals in the community or in non-psychiatric departments, which can be sub-divided into three scales of anxiety, depression and somatic symptoms. (See page 8)

9. The SCID was not administered this is a limitation that should be discussed.
Answer: As the reviewer pointed out, we did not use SCID in our diagnostic process, however, the Mini-international Neuropsychiatric Interview (MINI) was used (As added in the sample collection section on page 7). The MINI is a structured diagnostic interview which complies with the diagnostic criteria of DSM-IV and ICD-10.

References


The manuscript is revised, and resubmitted to BMC Psychiatry. If you have other comments or need further information, please let us know.

Best Regards,

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