Author's response to reviews

Title: Association study between SNP rs150689919 in the DNA demethylation gene, TET1, and Parkinson's disease in the Chinese Han population

Authors:

Xin-xin Liao (xinliao_86@126.com)
Zi-xiong Zhan (zhanzixiong@163.com)
Ying-ying Luo (luoyingying08@yahoo.com.cn)
Kai Li (liskai@126.com)
Jun-ling Wang (wjling8002@126.com)
Ji-feng Guo (guojifeng2003@yahoo.com.cn)
Xin-xiang Yan (xxyan1268@yahoo.com.cn)
Kun Xia (xiakun@sklmg.edu.cn)
Bei-sha Tang (bstang7398@163.com)
Lu Shen (shenlu2505@126.com)

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Author's response to reviews: see over
Dear Julie Anne Magtira,

We would like to thank BioMed Central for giving us the opportunity to revise our manuscript (Manuscript ID: 3293438011013600, Association study between SNP rs150689919 in the DNA demethylation gene, TET1, and Parkinson's disease in the Chinese Han population.), and thank the reviewers for their careful read and thoughtful comments on previous draft. We have studied the comments carefully and have made deeply correction which we hope meet with approval. The following is our response to the reviewer’s comments point-by-point.

Reviewer Dr. Hiroyuki Tomiyama:

1. **Comment:** I feel discussion section may be a little bit redundant.

   **Answer:** Thanks for the reviewer’s good advices. The Discussion section has been re-written in revised manuscript. The sentences states the role of TET1 was deleted, while previous reports on rare variants in PD and the tentative approach to detect rare variants for complex diseases were discussed.

2. **Comment:** The authors should show pedigree trees of the families with TET1 rs150689919 because this study is started from their family based study conducted by exome sequencing.

   **Answer:** Thanks for the reviewer’s good suggestions. The pedigree trees of the families with TET1 rs150689919 were shown as follow, which have been added in the revised manuscript as Additional file 2. The variant c.1460C>T in the TET1 gene (rs150689919) was only existed in three unrelated cases from three PD families and not segregated with the disease status.
Supplement Figure 1. Pedigrees with variant TET1 rs150689919. Each proband was indicated by an arrow. Patient II:2 in Family M17306, patient II:2 in Family M8302 and patient II:3 in Family M13742 shared the variant TET1 rs150689919.

3. **Comment:** If the family members have other variants or mutations in the TET1 gene, please provide the analyzed members data by the exome analysis in a table. This may disclose whether there is less possibility of association between sporadic PD and the TET1 gene or not in the Chinese population.

**Answer:** Thanks for the reviewer’s good advice. Actually, the family members have other variants in the TET1 gene, that is, three SNPs in TET1 were called per individual on average, but no indel had been found by exome sequencing. In order to select more marked functional variants, the SNPs located in introns or with the population MAFs>5% were removed. Taken together, the SNP rs150689919 was the only one qualified variant in TET1 gene, showing the highest frequency in the sixteen patients.

**Reviewer Dr. Georgios Hadjigeorgiou:**

1. **Comment:** First paragraph. Authors present a typical candidate gene association study for Parkinson’s disease (PD) based on data from exome sequencing from their group. Authors referred to "our previous work ...." for exome sequencing albeit they did not cite any paper for this work. If these are unpublished data then they have to provide more data from exome sequencing in familial cases.

**Answer:** Thanks for the reviewer’s good suggestion. Actually, “our previous work” on exome sequencing is still unpublished; therefore, we re-wrote the Background
section and added the detail screen strategy in the revised manuscript. In addition, the candidate variants from exome sequencing of eight PD families were also listed in Additional file 1.

2. **Comment:** Second paragraph. Authors stated: "the variant c.1460C>T in the TET1 gene (rs150689919) as the most promising". Please provide a convincing etiology for this.

   **Answer:** Thanks for the reviewer’s good advice. The reasons of choosing c.1460C>T in the DNA demethylation gene, *TET1* as the most promising variant have been rewritten in the Background section. Briefly, according to the significant roles epigenetics playing in the pathogenesis of PD and the relatively high frequency of rs150689919 in our familial PD cases, we chose it as our candidate variant.

3. **Comment:** Authors reported age- and gender- matched controls. How did they performed matching: By one-to-one, by 5- or 10- years interval? It is quite strange that the standard deviation for age for PD patients is double that of controls (albeit mean age is similar). Please provide an explanation.

   **Answer:** Thanks for the reviewer’s comments. We collected all the patients who met the inclusion criteria during investigation, and all controls were healthy people from Physical Examination Center of Xiangya Hospital with no history of neurodegenerative disorders and other diseases. According to the age distribution of the patients group (at the interval of 10 years), we selected the age- and gender-matched healthy controls. We didn’t perform the matching by one to one, but for each age interval, we just selected approximately equal number of gender-matched subjects as the control group. Consequently, the standard deviation for patients with PD was double that of controls. Therefore, we considered that the difference of the standard deviation of these two groups did not affect the results of the experiment.

4. **Comment:** Were controls and patients consecutive cases? Were hospital or population based? The clinical status of controls was based on interview, on files or
on clinical evaluation? Please try to better define controls for neurodegenerative diseases like Alzheimer's disease, other extrapyramidal diseases apart from PD in order to minimize the possible for selection bias.

**Answer:** Thanks for the reviewer’s good advices. Both of the patients and the controls are consecutive cases. The patients with PD were recruited from the Department of Neurology, Xiangya Hospital and the Key Laboratory of Neurodegenerative Disorders in Hunan Province from October, 2005 to March, 2012. All the patients were evaluated by two experienced neurologists and diagnosed as idiopathic PD based on the United Kingdom Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria. During the same period, unrelated subjects from Physical Examination Center of Xiangya Hospital were evaluated by two experienced neurologists, and healthy controls free from signs of Parkinsonism, Alzheimer's disease and other extrapyramidal diseases were enrolled in our case-control study matched for age, gender, ethnicity, and area of residence. We have better defined the controls in the “subjects” part of Method section.

5. **Comment:** Please provide a power analysis in "Statistical analysis". Authors recognize that their study is underpowered in Discussion albeit they did not provide a power analysis.

**Answer:** Thanks for the reviewer’s correction. We are very sorry about that. The statement of the sentence may be a little bit obscure. We didn’t mean that the statistical analysis was underpowered, and just want to say that select candidate rare variants from the exome sequences was underpowered due to the limitation of sample size and low frequencies of the risk alleles. In order to avoid misunderstanding, we have corrected the statement as “Second, when selecting candidate variants of complex disorders from exome sequences, it requires moderate sample size, or informative pedigrees to simplify the filtering of variants [15]. In this study, due to the limited sample size of the PD pedigrees and the rare frequencies of the risk alleles in the population, we failed to identify the risk variant for PD”.

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6. **Comment:** Most of the Discussion is not relevant to the study. Authors have to re-write the Discussion and discuss their results in comparison with other studies in the field of rare variants in PD. The role of TET1 gene has already covered in "Background". Please delete second paragraph. It is not relevant.

**Answer:** Thanks for the reviewer’s suggestion. The Discussion section has been re-written in the revised manuscript. The sentences states the role of TET1 was deleted, while previous reports on rare variants in PD and the tentative approach to detect rare variants for complex diseases were discussed.

7. **Comment:** First paragraph. Last sentence: "Genetic association studies based on the “candidate gene approach” and genome-wide association studies have revealed several genetic variants that might act as susceptibility factors for the sporadic cases [3-5]". Reference 3 and 4 are studies in Chinese population and this has to be recognized by the authors. I suggest to cite PD gene (www.pdgene.org) for those who are interested to check all studies in the field. Similarly in second paragraph.

**Answer:** Thanks for the reviewer’s correction. We have cited PD gene database (www.pdgene.org) instead of the previous documents in the revised manuscript.

8. **Comment:** Second paragraph. Please define "PolyPhen".

**Answer:** Thanks for the reviewer’s comments. We already defined PolyPhen and added the URL of the tool in the revision manuscript.

**Reviewer Dr. Alessio Di Fonzo:**

1. **Comment:** Is the “previous” exome-sequencing study on the eight Chinese families an unpublished work? The authors might specify this or add a proper reference.

**Answer:** Thanks for the reviewer’s good suggestion. Actually, “our previous work” on exome sequencing is still unpublished; therefore, we re-wrote the Background section and added the detail screen strategy in the revised manuscript. In addition, the candidate variants from exome sequencing of eight PD families were also listed in see Additional file 1.
2. **Comment:** If the exome sequencing data are new, I suggest to move the paragraphs related to that from the Background to the Methods and Results sections.

**Answer:** Thanks for the reviewer’s suggestion. The exome sequencing data in the Background was the foundation of the research, and clarified why we focused on rs150689919 in *TET1* gene. Consequently, we prefer to state it at the beginning of the study instead of move to the Methods and Results sections.

3. **Comment:** Is it known whether the rs150689919 SNP cosegregates with PD among the three families carrying the variant?

**Answer:** Thanks for the reviewer’s comments. The SNP rs150689919 wasn’t co-segregated with PD among the three families carrying this variant. Because the purposes of our study is to find the susceptibility factors for sporadic cases, we focused on the variants shared the highest frequency in 16 patients instead of the variants segregated with the disease status. On the other hand, it is also a wonderful idea to identify the association between causative mutations for monogenic forms and sporadic cases; however, we didn’t found such variant from these families till now.

We appreciate for Editors/Reviewers’ warm work earnestly, and hope that the correction will meet with approval. Once again, thank you very much for your comments and suggestions.

Thanks and Best regards!

Yours sincerely,

Lu Shen
Department of Neurology
Xiangya Hospital, Central South University
87 Xiangya Rd, Hunan 410008, P.R. China
Tel: 86-731-84327632
Fax: 86-731-84327332
Email:shenlu2505@126.com