Author's response to reviews

Title: Lack of association between PRNP 1368 polymorphism and Alzheimer's disease or vascular dementia

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Author's response to reviews: see over
Dear Prof. Scott Edmunds

Thank you very much for forwarding the review of our manuscript entitled “Lack of association between PRNP 1368 polymorphism and Alzheimer’s disease or vascular dementia”. We have revised the manuscript in accordance with the suggestions made by the editor and two reviewers and are therefore submitting the revised manuscript.

In relation to the comments of the editor and two reviewers, our corrections and revisions are as follows:

**Editor**

In particular there needs to be more justification for the rationale and stressing the advance of the study (such as why the results were not combined with the 1360 polymorphism).

**Response:** According to the editor and reviewer’s suggestion, we have added information to the Discussion about the rationale of the study and the potential for further work.

Please also ensure that your revised manuscript conforms to the journal style (http://www.biomedcentral.com/info/ifora/medicine_journals). It is important that your files are correctly formatted.

**Response:** As suggested by the editor, our manuscript was correctly formatted according to the journal style.

We recommend that you ask a native English speaking colleague to help you copyedit the paper.

**Response:** Two native speakers re-edited our manuscript.

**Reviewer: 1**
1. Line 8-12: this sentence is hard to follow, and maybe interpreted wrongly by readers. **Response:** According to the reviewer’s suggestion, we have changed the sentence in the Background section [page 3, lines 16-18].

2. Line 18-19: what do the authors mean by the second part of the sentence: ‘including a role for oxidative stress and the occurrence of dementia’. This part does not seem to be related to the first part of the sentence. It is not clear what it means. **Response:** As suggested by the reviewer, we have clarified the sentence in the Background section [page 3, lines 23-24].

3. Line 13: ‘In a British..’ the link with previous sentences is missing. This sentence appears out of the blue. **Response:** To naturally link the two sentences, we have added the following sentence: “This polymorphism was studied in other diseases in addition to sporadic CJD” to the Background section [page 4, line 13].

4. The authors did not describe how they ascertained their cases, there is no description on where the patients where seen, who diagnosed them, whether the researchers verified the diagnosis, examined them or only took blood, and in which period they were seen, etc. The authors should describe this more extensively. Now it is not clear what procedures were followed. **Response:** We have added the detailed information about patients to the Methods section [page 5, lines 7-14 and 19-23].

5. The authors do not report on the models they used in their analyses. It is not clear for which covariates they adjusted, and what models they applied. The authors should add this to the description on statistical analyses. **Response:** As suggested by the reviewer, we have added the information about controlled covariates and applied models in the Methods section [page 7, lines 9-11].

6. Also, the authors do not describe the haplotype analysis, while this is important information to the methods. **Response:** We have added the method of haplotype analysis to the Methods section [page 7, lines 12-13].

7. The authors don’t speculate on the reason why they didn’t find an association. While
it would add more strength to the discussion if they did.

Response: There is the possibility that the PRNP 1368 polymorphism is not functional with regard to affecting the level of PrP^C. Another possibility is that a false negative result was obtained due to statistical powers. Data for AD and VaD patients showed a statistical power of 19.1% and 11.2%, respectively, at the Type I error rate of 0.05 compared with healthy controls. The statistical powers aren’t high enough for ensuring that the PRNP 1368 polymorphism is not relevant to prion replication. However, in the haplotype analysis among 3 PRNP polymorphisms, haplotype ht5 was the only haplotype significantly associated with VaD (p=0.013) (Table 3) and the genotype frequency of PRNP 1368 polymorphism in VaD patients was not in HWE. These results suggested some interaction among 3 PRNP polymorphisms in the determination of VaD risk and were needed for further evaluation of the association of PRNP 1368 polymorphism with VaD in other ethnic groups. This information has been added to the Discussion section [page 8, lines 16-24; page 9, lines 1-6].

8. The authors should add a power calculation.
Response: We have added the method and results of power calculation to the Methods section [page 7, lines 15-17] and the Discussion section [page 8, lines 21-23].

9. The last paragraph on page 11 appears just like that. There is no link to previous paragraphs.
Response: As suggested by the reviewer, we have deleted the paragraph in the Discussion section [page 10].

10. Line 5: please change ‘another’ in ‘other’
Response: We have changed “another” to “other” in the Abstract section [page 2, line 6].

11. Line 6-8: The idea behind this sentence is not clear. It would aid if this sentence was introduced in the previous sentence.
Response: This paragraph has been rewritten so that there is now an effective background and introduction to our planned work [page 2, lines 2-10].

12. Line 3: it is a very brief description of the clinical characteristic of AD. This can be
discussed more extensively.

**Response:** As suggested by the reviewer, we have added extensive information about AD to Background section [page 3, lines 1-9].

13. Line 14: can easily be omitted.

**Response:** We have deleted the sentence in the Background section [page 3, line 20].

14. Line 16: second part: ‘the association study between..’ can be formulated differently, it is not clear what the authors want to state here.

**Response:** As suggested by the reviewer, we noted that we used a case-controlled study to examine the association between PRNP 1368 and the 2 diseases of interest. This is in the Background section [page 4, lines 16-18].

15. Line 20: the authors do not say in which group (patients or controls) the genotype were in HWE. This would be helpful in this part.

**Response:** We had stated the results concerning HWE in the Results section. This is noted on page 7 [lines 20-21].

16. Line 8-10: the conclusion in this sentence does not fit the results. They authors stratified on gender, and conclude that there was no association between the polymorphism and gender, while in fact they did not test this.

**Response:** According to the reviewer’s suggestion, we have corrected the sentence “no association between the polymorphism and gender” to “no association between the polymorphism and AD” in the Results section [page 8, line 4].

17. Line 17-18: The authors mention that 1 haplotype was associated with VaD, but in the next sentence they conclude that the PRNP 1387 polymorphism was not associated with the disease. It is not clear on the basis of which findings, the authors conclude this. The sentence can be deleted here, and pasted to the conclusions.

**Response:** According to the reviewer’s suggestion, the sentence “These results suggest that the PRNP 1368 polymorphism is not associated with the occurrence of VaD in the Korean population” has been deleted in Results section [page 8, line 13]. This sentence has been reworded and moved to the Conclusion section [page 10, lines 17-19].
18. Page 10, Line 2: which mechanisms do the authors mean. Genetic mechanisms?
   **Response:** As suggested by the reviewer 2, this sentence was deleted in Discussion section to avoid repeating in Background and Discussion.

19. Page 10, Line 4: please change ‘has’ into ‘have’
   **Response:** As suggested by the reviewer 2, this word was deleted in the Discussion section to avoid repeating in the Background and Discussion sections.

20. Page 10, Line 19-20: ‘recently, there has been growing ..’ It would be better if the authors would explain why there is growing concern. Has it been reported in other studies? Has it been linked to AD before? What might be the pathway?
   **Response:** There has been growing concern about several polymorphisms outside the ORF of PRNP, as there is evidence that levels of PRNP expression influence incubation time and the susceptibility to prion diseases: added on page 9, bottom paragraph.

21. It is not clear why the authors choose the 1386 polymorphism to study, when there is so little evidence from other studies. What is their hypothesis on this polymorphism? What role can it play? Why would this be the most important one, and what role does it play, compared to other polymorphisms?
   **Response:** As suggested by the reviewer, we have added the information for the hypothesis, role, and the reason for selecting the 1368 polymorphism in the first paragraph of the Abstract, the first paragraph on page 4 and in the last paragraph on page 9, first paragraph on page 10.

22. It would be of additional value if the authors would speculate on the possible implications of this study to future work? And how to proceed form here?
   **Response:** We have added the information for future work in the Discussion section [page 10, lines 12-14].

23. Tables 2 and 3 can be combined.
   **Response:** According to the reviewer’s suggestion, Tables 2 and 3 have been merged. All information has been incorporated into Table 2.

24. The authors should add a legend with information on the numbers that are depicted in the tables.
Response: We have added the information on the numbers to Tables 1 and 2.

25. Abbreviations are different between tables, please make them consistent, and add a legend with the meaning of these.
Response: We have added the information on Abbreviations to Table 2 and unified the Abbreviations in Table 3.

Reviewer: 2

Major Compulsory Revisions
1. The Authors should avoid repeating in the “Discussion” what they have already described in the “Background”. For example, first paragraph of “Discussion” and first/second paragraphs of “Background”, second paragraph of “Discussion” and third paragraph of “Background”. It would be preferable to have a comprehensive description of the state of art of the problem in the “Background” and use the “Discussion” for an in-depth analyses of the data and for constructive speculations.
Response: According to the reviewer’s suggestion, we have omitted repeating sentences in the Background section [page 5, lines 6-11 in submitted form] and the Discussion section [page 10, lines 2-9 and page 11, lines 1-7 in submitted form].

2. The Authors should comment on their only positive results (see table 4) and on the absence of the HWE for VaD patients
Response: As suggested by the reviewer, we have added the information about one positive result and absence of HWE in VaD to the Discussion section [First paragraph, top of page 9].

Minor essential revisions:
1. Abstract. PRNP should be in italics.
Response: This has been corrected where appropriate in the Abstract section [page 2].

2. Background, page 1, first paragraph: “…senile plaques commonly contain PrP deposit in AD…” . Which kind of PrP? PrPc or PrPres? Reference 2, for example refers only to PrPc.
Response: We have changed “PrP” to “PrP\text{C}” in the Background section [page 3,
3. Background, page 5. The polymorphic codon 219 of PRNP is a susceptible factor to sporadic CJD only in the Asiatic population (see Petraroli and Pocchiari, Am J Hum Genet 1996).

**Response:** We have added the reference to the Background section [page 4, line 5] and the Reference section [page 12, reference 8].

4. Methods, page 6. The Authors should clarify whether AD patients were sporadic or familial. The Authors should also provide statistical evidence that controls, AD, and VaD patients were matched for age and sex.

**Response:** None of these patients reported family history of AD. Differences in sex between controls and AD or VaD patients were verified using the $\chi^2$ test. Age values were analyzed by Student’s $t$-test. This information has been added to the Methods section [page 5, line 9 and page 7, lines 11-12] and Table 1. Although there is a significant difference in the mean age of AD patients and controls, a difference of less than 3 years certainly qualifies as age-matched.

In conclusion, we have addressed all of the comments raised by the editor and two reviewers. We believe that the changes suggested by the reviewers have improved the manuscript, and we hope that it is now acceptable for publication in “BMC Medical Genetics”.

Very sincerely yours,

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