Author's response to reviews

Title: The association of APOE genotype and cognitive decline in interaction with risk factors in a 65-69 year old community sample

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Author's response to reviews: see over
Dear Mr Kerr,

We are submitting a revised copy of our manuscript (#9052766791860029), “The association of APOE genotype and cognitive decline in interaction with risk factors in a 65-69 year old community sample”. Changed text has been highlighted in the document. A point-by-point description of the changes made is below, with reference to the reviewer’s comments. Please do not hesitate to contact me if you require anything further.

Sincerely,

Helen Christensen

Reviewer: Janet Johnston

Major Compulsory Revisions

1. A reference should be added for the Reaction test, as well as for the method used for handling outliers.
   We have added a reference for the Reaction Time tests that details the procedure and forms the basis for how the outliers were handled (see p. 9: Reference [26])

2. What statistical tests were used for Table 1? It would be best to use a test for trend to test whether there is a greater effect with two E4 alleles as opposed to one.
   We have added a sentence in the results section clarifying the statistical tests used in Table 1 (p.11).

3. I was not clear exactly how the clinical diagnosis was used as an exclusion criteria. Was everyone with any diagnosis of mild cognitive impairment, age associated memory impairment, age associated cognitive decline, or mild neurocognitive disorder excluded when the analyses were repeated. And what happened when they were excluded. I do not see two sets of results (one with the 127 people with clinical diagnosis and one without) reported.
   Every participant with any of the above diagnoses was excluded from the follow-up analysis. A sentence has been modified in the method section to clarify this exclusion (p. 11). The two sets of results are in the table (was Table 3, now Table 2) – Section A shows the results for all participants while Section B shows the results with the exclusion of the 126 people with a diagnosis.

4. Please explain how missing data was handled.
   Two additional sentences have been added to the Analysis section of the method to explain the extent of missing data and how they were handled (p. 10-11).

5. Please provide more details regarding model fitting and what diagnostics were used.
   A sentence has been added to the results section detailing the range of R² values for the seven ANOVA models presented. All of the R² values are very small, reinforcing the limitations we have placed on our findings. (See p. 13.)

6. Please comment on whether the APOE genotype was in Hardy-Weinberg equilibrium.
   We have added Hardy-Weinberg equilibrium statistics to the method section (p. 7).
7. I found the last two sentences of the Bivariate models paragraph in the Results section to be confusing. The second to last sentence talks about those homozygous for *E4 while the last sentence talks about those homozygous or heterozygous for *E4. From Table 2 the reader can only tell that there was some effect of the APOE genotype, but cannot tell anything about differences between homozygotes and heterozygotes. I would prefer to see regression coefficients and 95% confidence intervals for the significant models, rather than a table of mostly non-significant statistical test values and p-values. We have removed the bivariate models table from the paper, as suggested by both reviewers. We concur that the table did not add much to the paper. The sentences have consequently been removed.

8. In Figure 1, the error bars for the *E4 homozygotes in panels C and D include 0, making me question your statement that they showed greater decrease in MMSE and SDMT than all other groups. It is true that the genotype/head injury interaction was statistically significant, but the figure does not show a significant interaction for that group. We have combined the homozygotes with the heterozygotes for Figure 1 for simplification.

9. The authors provide a relatively long introduction with a good review of the literature; however, they then do not talk about how the results fit into the literature in the discussion. I recommend shortening the introduction and including more information about other studies and where these results fit in the discussion. The discussion and the introduction have been redistributed to accommodate this request for revision.

**Discretionary Revisions**

10. It would have been nice to be able to distinguish between stroke and TIAs in the analysis. Given that only one question was asked, it does not seem possible to change that for this analysis. However, for the future I would recommend getting more detailed information about stroke vs. TIA. We agree with the reviewer that future studies should distinguish between stroke and TIA.

11. The cutpoints for hypertension seem high, particularly given current hypertension definition of SBP $>=$ 140 and DBP $>=$ 90. Would consider re-doing analysis with lower cutpoints. We redid the analysis with the lower cutpoints recommended. The effect of hypertension remained non-significant for all of the cognitive tests. We have used these new cutpoints in the paper (see p. 8).

12. Because of the relatively small number of participants who are homozygous for the E4 allele, the authors may want to consider dichotomous analyses where the two groups are no E4 allele vs any E4 allele. This might help shrink the confidence intervals when you start looking at interactions. We reran the analyses combining homozygous and heterozygous participants as suggested. This analysis resulted in a similar pattern of results, albeit with slightly more significant effects. However, we chose to retain the split between homozygotes and heterozygotes, as there are relatively few studies available which have sufficient power to study this distinction. For the graphs of the effects in Figure 1, we did collapse groups for simplicity.
13. I would divide Table 1 into two tables, either Table 1 and Table 2 or Table 1A and 1B, with the Wave 2 test scores and change in test scores in the second table. I would also leave out the value of the test statistic and just include the p-value. The tables are very busy, making them a little overwhelming to read. We have divided Table 1 into 1A and 1B and removed the test scores on the advice of the reviewer. We agree that concisely presenting a large number of descriptives can be problematical.

14. In many longitudinal studies of cognitive function there is a learning effect where scores may actually increase the second time a test is given. I find it interesting that almost all of the test scores went down from Wave 1 to Wave 2. This may be due to the age of your participants. You might want to comment on this in the discussion. If you have completed Wave 2 for your younger groups of participants, it would be interesting to know whether the scores also fell from Wave 1 to Wave 2 for those groups. We have previously seen this effect in other longitudinal studies we have conducted. It is an interesting point. The decrease in cognitive test scores was only seen in the 60s age group, with the exception of SDMT which decreased non-significantly among the 40s age group. Most of these tests (with the exception of the memory tests) are unlikely to show a learning effect in the four years from Wave 1 to Wave 2. This is an issue to pursue in the future.

15. I think it is a good idea to use figures to show the effect of the interactions, however, I think the figures would be easier to read if you flipped the groupings. For example, I would have had an easier time reading the figures if in panel A the first group was everyone with education 0 to 12 years and then there were 3 bars in that group showing the different APOE genotypes. We have flipped the groupings as suggested, which makes the figure clearer particularly after combining the heterozygotes and homozygotes.

16. In looking at Figure 1, there are many instances where there are large error bars that include zero, especially for the E4 homozygotes. Again, I would suggest combining the heterozygotes and homozygotes in hopes of shrinking the error bars and being able to report more robust findings. We have combined the heterozygotes and homozygotes for Figure 1 as suggested.
Reviewer: Ian Deary

Major compulsory revisions

1. I don’t think that both the bivariate and multivariate models are necessary. One learns little new from the latter after the former. It could merely be stated that these were done and what, if anything, new emerged. We concur with the reviewer’s suggestion and have removed the bivariate table. As we used the bivariate models to guide the multivariate models, we thought it appropriate to retain the multivariate models. The presentation of the multivariate table is also neater than the bivariate table.
2. Can the authors please check that they have indeed used standard error bars in the panels in Figure 1? With the very large error bars it is hard to see how any of these effects could have been significant and one wondered if some were standard deviations. The bars were standard error bars. The size was due to there being very few participants in some of the subgroups (e.g., only four homozygous participants reported head injury). We have consequently combined the homozygous and heterozygous participants for Figure 1.
3. Eta squared values should be included for the significant results, so that readers can have an idea of the effect sizes. We have included the range of eta squared values, in the results section. All of the eta squared values are very small, reinforcing the limitations we have placed on our findings. (See p. 13.)

Minor essential revisions

4. Would the authors please report on Hardy Weinberg equilibrium, both at baseline and 4-year follow-up? We have added Hardy-Weinberg equilibrium statistics to the method section. (See p. 7.)

Competing interests, authors’ contributions and acknowledgements
These have been moved from after the reference section to before the tables, as requested.