Reviewer’s report

Title: Effects of Homocysteine on White Matter Diffusion Parameters in Alzheimer's Disease

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Reviewer: Barbara B. Bendlin

Reviewer's report:

This paper details the results of an analysis examining the relationship between plasma homocysteine levels and measures of white matter microstructure as assessed with diffusion tensor imaging among 132 participants with dementia due to Alzheimer's disease. The investigators also measured the relationships between homocysteine and cognitive function, as well as DTI metrics and cognitive function. The results showed no effect of homocysteine on cognitive function. Plasma homocysteine levels were associated with white matter imaging parameters including FA, MD, RD, and AD. Finally, the investigators also report that homocysteine levels are associated with renal dysfunction, lower B12, and lower folate levels.

Strengths of the study include the relatively large sample size, and the careful neuroimaging analysis. Homocysteine levels were shown to be associated with white matter microstructure, which is an interesting finding. The results are presented clearly in tables, and in figures. At the same time, there are several ways that the manuscript could be improved; presently, the writing is unclear and there are many grammatical errors. The overall motivation for the study is not adequately presented, and the results of analyses that appear peripheral to the primary objective of the study are not well explained (for example the relationship between homocysteine and renal dysfunction). Additional questions and comments are as follows:

1. The introduction discusses white matter hyperintensities (WMH) and the investigators suggest that they will examine WMH in this study (as stated on pg. five: "we hypothesized that hyperhomocysteinemia in late-onset AD may lead to a greater WMH loads"). However, WMH are typically evaluated using T2FLAIR weighted imaging (or T2 and PD), not DTI. In this study, the investigators are examining white matter microstructure; this should be corrected.

2. The investigators conclude that "homocysteine levels reflected renal dysfunction status and decreased vitamin B12 and folate state that required clinical attention as they may associated with impaired WM microstructural integrity and modulate the cognitive performance in cross sectional observation". I do not believe this is shown in the current study. There were no main effects or interaction effects of homocysteine on cognitive function. Again, in the discussion section, the paper states that "Randomized, controlled and longitudinal follow-up studies are warranted to clarify whether the homocysteine lowering treatment can improve
the cognitive outcomes in the future”. In the current study, there does not appear to be an effect of homocysteine on cognitive function.

3. Were any data lost or excluded? In a study of AD, it would not be uncommon that some participant data are excluded due to motion or other challenges in scanning this population.

4. APOE4 genotype was determined but not included in the analysis. Prior studies suggest APOE4 genotype may be associated with homocysteine levels, this may be a factor to consider here.

5. Can the authors provide their rational for conducting both a TBSS analysis, and ROI analysis (i.e. tractography analysis)? The analyses use the same underlying data, so it may be sufficient to present just one set of analyses.

6. The statistics section indicates that SPSS was used for all statistical analysis. This should be clarified to indicate that FSL was used for voxel-wise analysis.

7. Given that FA, MD, RD, and AD were assessed in 11 ROIs, and used to predict several cognitive scores, and as well as tested for associations with several lab values, should the authors perhaps consider correcting the ROI analyses for multiple comparisons as well?

8. What was the purpose of conducting the analyses with and without covariates (confounders), for example, as presented in pages 10-11? Was the idea to determine whether these factors mediate the relationship between homocysteine and white matter microstructure? The motivation for various analyses are not adequately described, and the results not fully discussed.

9. The first paragraph of the abstract is not clear and could be improved.

10. The conclusion that the findings are consistent with data on the methylenetetrahydrofolatereductase gene should be explained.

11. Grammatical errors need to be addressed throughout the manuscript.

Minor:

1. The authors might consider leaving out the word "different" in the title.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes
Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
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Not suitable for publication unless extensively edited

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