Author's response to reviews

Title: Alzheimer's disease: structural and cognitive basis of odor identification deficits

Authors:

Grete Kjelvik (kjelvik@ntnu.no)
Ingvild Saltvedt (ingvild.saltvedt@ntnu.no)
Linda R. White (linda.white@ntnu.no)
Pål Stenumgård (Pal.Stenumgard@stolav.no)
Olav Sletvold (olav.sletvold@ntnu.no)
Knut Engedal (knut.engedal@aldringoghelse.no)
Kristina Skåtun (kristinaskatun@gmail.com)
Ann Kristin Lyngvær (ann.kristin.lyngver@stolav.no)
Hill Aina Steffenach (hillaina@gmail.com)
Asta K Håberg (asta.haberg@ntnu.no)

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Author's response to reviews: see over
Dear Jhonell De Los Santo,

Thank you very much for your e-mail with links to the reviewers’ comments, as well as the extension to allow for vacations and other travel. We are grateful for the opportunity to reply to the reviewers for their comments and will try to address them systematically. Please see our comments and modifications below.

Reviewer: Martijn Muller
Reviewer's report:

Major Compulsory Revisions
Impaired olfaction has been well-documented in both prodromal and disease stages of Alzheimer disease (AD) and Parkinson disease (PD); especially the impaired ability to identify various odors. Frequently used methods to test for odor identification impairment include the University of Pennsylvania Smell Identification Test (UPSIT) and the 'Sniffin Sticks' Identification Test (SSIT). The authors note that some AD and MCI patients have relatively intact odor identification abilities. This observation forms the premise of this paper. Cognitive and brain volumetric measures are compared between patients with intact vs. impaired odor identification function. My main concern revolves on the criteria of "intact" and "impaired". For example, for the brief version of the UPSIT (B-SIT) the authors base the cutoff on criteria used by Westervelt et al. (2007), who applied a 'median split' on the possible score range of the test (0-12); i.e. 7 or better ("intact") vs. 6 or less ("impaired"). In my opinion, this is an incorrect and arbitrary approach and the cutoff should really be based on the median scores of the normal control population. Figure 1 of this paper provides some insight in the performance of patients on the two odor identification tests. The median scores of the normal controls for the BSIT and SSIT appear to be at 10 and 13, respectively. Based on these cutoff criteria there are only 2 "intact" odor identification patients. I believe that my estimate is more in line with previous literature (for example Gray et al (2001) and Westervelt et al (2008) which both were cited by the authors) than the estimate based on the current cutoff. Actually, Gray et al. show complete separation between AD patients and normal control subjects based on this approach (figure 1 of their paper).

It can be a lengthy and perhaps impossible discussion to decide on criteria for 'normal' odor identification performance in an older population. However, the authors should provide a better justification for the cutoff criteria that they used and especially make clear why they do not base this on their own normal control data. On this note, it should also be noted that there are 2 'normal' controls with extremely low odor identification scores (figure 1). If peripheral factors can be excluded as an explanation for these low scores, the authors should consider that these two subjects may have (prodromal) synucleinopathy. With a small group this may skew the results.

We are particularly grateful to Dr. Muller for pointing at that our use of 'intact' versus 'impaired' is incorrect. On reflection we agree that these are not suitable terms at all. All participants in the study are old, and some controls of course also have OI ability below their group median. Indeed, it would be odd to have a cohort of 30 elderly participants healthy for their age without one or two individuals showing signs of reduced cognition (as is the case in our control group). We also agree that the best way to distinguish the patients as a whole from the controls would be to compare values against the control group median. However, this is not really the main aim in our paper.

The problem we agree has been largely created by our terminology of 'intact' and 'impaired' OI. Since the main aim in this study has been to compare changes in the volume of brain areas with OI abilities, as well as changes in memory, patients have been compared based on such changes. We also wished to see if the results were similar in two separate OI tests, but this requires establishing a cut-off for each that should bear a relationship to each other. Such cut-offs are not necessarily at the
lower end of normal OI (as the term “intact” inferred), but rather where each test reflects a similar change in OI ability (those with intact or some OI, as opposed those with little or no OI ability).

We still wish to divide our patients in this way regarding OI ability, and have not changed the distribution of patients as previously classified. Setting a cut-off at 50% admittedly is arbitrary, but those with 50% scores or less will probably have a compromised OI, and it is unlikely that patients will achieve over 50% on both these tests purely by chance (achieving 7 correct answers on B-SIT by chance is p=0.011, and achieving 9 correct answers on SSIT by chance is p=0.006). The text has been modified in a number of ways as indicated. Particularly, the terms 'intact' and 'impaired' have been removed, and replaced in tables by >50% or ≤50%. The data have been extensively checked. We apologise but there was a mistake in the calculation of the kappa, sensitivity and specificity values for SSIT and these data have been corrected.

In conclusion, if the approach laid out in this paper would not be changed, the results and conclusions presented in this paper appear to be valid although underpowered overall. However, as explained, I see problems with the criteria used for defining impaired and intact odor identification, especially since it is not based on cutoff of their own normal control data. If the authors would follow my recommendations, a larger group would be needed to find more "intact" MCI/AD subjects to achieve appropriate statistical power.

We would gladly have included more individuals, but it is clear from the Methods that a great deal of effort and patience was required on their part to fulfil all aspects of the study. Consequently by the end, a larger number than expected had to be excluded for a range of reasons, mainly failure to meet all inclusion criteria (which were stringent) or unwillingness to complete sufficient tests.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

These changes have made a substantial contribution to the overall concept of the patient OI division, which was both welcome and necessary.
Reviewer: Tibor Kovács

Reviewer's report:

Major Revisions

1. The authors state that no tests were administered at follow-up although this information could have been useful to interpret the results.

We had no permission from our IRB to conduct research tests at follow-up, which was conducted on a purely clinical basis.

2. The conversion rate of aMCI to AD during the follow-up is high (7 out of 12), especially taking the short follow-up duration (6-18 months) in consideration (no mean of follow-up is given). In addition, aMCI and AD patients at baseline had no differences in cognitive tests, except RCFT copying, which is clinically rather unusual and probably might influence the main message of the paper.

The mean of follow-up was 9.9 months, and this has been added to the text. That so many patients converted from aMCI to AD in a short space of time is perhaps also the explanation as to why there were so few statistical differences between the aMCI and patients with AD for the cognitive tests. A higher conversion rate should also be expected when only patients with amnestic MCI are included, rather than a mixed group including non-amnestic MCI. Another cohort, younger on average by 10 years, followed by us longitudinally over 2 years also shows a high percentage of conversion (49%) within this period (unpublished results). In an older cohort, as in the present study, the period between aMCI being diagnosed and onset of dementia may well be accelerated as cognitive reserve declines with increasing age. This has been added to the Discussion.

A discussion of the result with the RCFT has been added to the discussion (p. 19).

3. The authors analysed structures "considered to be involved in olfaction", such as total volume of hemispheric white matter, cortical grey matter, thalamus, total ventricular volume (all of these are too rough to compare in initial cases of AD), and the hippocampus and amygdala (which are known to be altered in AD and their volume loss is related to olfactory deficits) and mention that they did not analysed the volume of the entorhinal cortex, or the olfactory bulb. These methodological problems should have been corrected before publication (manual tracing, secondary analysis).

The study used the clinical equipment and methods available. We used Neuroquant (http://www.cortechs.net/products/neuroquant.php), an FDA approved brain volumetric program that can be used in the clinic. Neuroquant is accessed directly from the patient archiving system (PACS), and in turn the results are sent back to the patient records. The program builds on Freesurfer. It would of course have been interesting to use voxel-based morphometry, or Freesurfer, in order to measure the volume of entorhinal cortex, or the olfactory bulb. These methodological problems should have been corrected before publication (manual tracing, secondary analysis).

4. Keeping in mind the very small number of patients, the significant differences in age and gender between the analysed groups is difficult to explain and interpret (such as a F/M ratio approximately 1:4 in SSIT "impaired Ol" group).

Patients were recruited consecutively for participation in the study, and controls recruited randomly. A number of individuals had to be ultimately excluded for diverse reasons, so the differences in gender (and perhaps age) between the groups may have arisen by chance in our opinion. Certainly it is important to correct for age. There is also a slight female preponderance of females in Alzheimer's disease, but women also consistently live longer. The data analyses have been corrected for age:
page 12 in the Methods, page 16 in the Results. We have not corrected for gender as we know of no study indicating that this influences the progression of Alzheimer's disease.

3. It needs an explanation what is the reason why the hippocampal volume correlates with MMSE in controls but not in patients, also the case with TWT delayed recall.

We have commented on these results in the Discussion, p.18.

Minor Revisions:

1. In the introduction, authors state that OI "can discriminate neurodegenerative diseases, such as... AD from normal ageing", and a little below that "OI ability is therefore not suitable for differential diagnoses".

This mismatch has been changed.


This paper has now been added.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Thank you, we really appreciate your feedback, and your suggestions about modifications to the manuscript.
Reviewer: Latha Velayudhan

Reviewer’s report:
The paper is well written, however I have number of concerns.

1. The paper is titled, 'Alzheimer’s disease: structural and cognitive basis of odor’. Unfortunately the abstract and paper do not convey this accurately. The paper explores association of cognitive measures and olfactory identification with structural brain measures, in aMCI and early AD subjects. The title needs to be amended.

We hope the new title is an improvement.

2. The sample size is very small: 6 AD patients combined with 12 aMCI patients. They are are then further classified into 'intact' and 'impaired' olfactory groups. It is not clear how many of them were AD or aMCI in the 'intact' and 'impaired' OI groups.

In the revised manuscript, 'intact' has been replaced with >50% and 'impaired' with ≤50% score on the respective test. Results from patients that remained stable with aMCI over the follow-up period, those that converted to AD, and those diagnosed with AD from baseline are noe given in the Results on p.14.

3. Entorhinal cortex is an important anatomical substrate implied in Alzheimer’s disease, cognition and olfaction. Unfortunately the paper doesn’t include this region at all.

As mentioned above (reviewer 2, point 3), this was due to lack of the necessary program at the time, and is in the text.

4. More details of the MRI structural measurements are required. Was it 1.5 T or 3 T MRI?

All MRI data were acquired at 3T. We are sorry this was for some reason left out of the text. This information has now been added to the Materials and Methods section.

5. Smoking can impair olfactory identification function. There are 8.3% current smokers under 'impaired OI' group under B-SIT. Was there any correction for smoking done in the analysis.

No correction was made for smoking as it amounted to a single patient only in connection with B-SIT. This patient did not complete SSIT. This is now considered in the Discussion, p. 19.

6. The limitations of the study need to be clearly stated.

The limitations are now considered in more detail throughout the Discussion.

7. Results and Discussion: There is no mention of testing association of olfactory identification with cognition (as the title of the paper implies), which is disappointing.

We have re-written the title to be more in keeping with what is described in the manuscript. We did not include so much data on the cognitive tests as our data were typical for results produced over many years. The data can of course be added if it is felt that they add to the overall content.

8. There have been number of neuroimaging and longitudinal studies, that support role of medial temporal lobe in olfactory deficits in AD and MCI, also cited by authors in the background. There is
little in this study that is novel. In discussion it is pertinent to discuss findings in the current study with previous known literature in this area.

A number of relevant papers have now been added to the references.

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Overall, the reviewers made many excellent comments that have substantially contributed to and improved the manuscript, and we are very grateful for their work. We hope the revised version is acceptable for publication in BMC Neurology.

Yours sincerely,

Asta K. Håberg, MD PhD