Author’s response to reviews

Title: Plasma long non-coding RNA BACE1 as a novel biomarker for diagnosis of Alzheimer disease

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Author’s response to reviews:

Dear editor

We are writing to re-submit the revised manuscript entitled “Plasma long non-coding RNA BACE1 as a novel biomarker for diagnosis of Alzheimer disease” (NURL-D-17-00245). I would like to take this opportunity to thank the reviewers to provide invaluable comments for the improvement of our manuscript. We have carefully studied the comments of the reviewers and made necessary changes in the revised manuscript. We hope that we have addressed all of the questions satisfactorily and the manuscript at current version can be accepted in your journal. I am looking forward to receiving your favor reply. Thank you very much.

Yours faithfully

John Ringman (Reviewer 1)
This is an interesting paper that looks at novel plasma biomarkers for AD. RNA is receiving increasing attention as a biomarker in AD and other diseases and this paper provides preliminary evidence for some utility of RNA for BACE1 in plasma being of diagnostic utility. Perhaps more importantly, the observation of increased RNA for BACE1 in plasma provides a window into the potential role of this enzyme in AD pathogenesis. A strength is that the control cohort were neuropathologically verified to be without AD or other pathology.

Major comments:

1. In the Introduction there should be some explanation for why BACE1, BC200, 51A and 17A were chosen to study. Other than BACE1, it is not stated what these other RNAs code for and the scientific rationale for choosing them.

RE: In this study, we did not screen the LncRNA by microarray analysis due to the funding limitation. These four genes were chosen due to the previous studies and literatures. We have added the explanation in the introduction section as follows:

Among them, BACE1 is essential for the production of the toxic Aβ and lead to the APP processing, which have a major role in AD. Therefore, BACE1 may be potential biomarkers and treatment targets for AD [16, 17]. One previous paper showed that 51A was a fresh LncRNA that maps in an antisense configuration to intron 1 of the neuronal sortilin-related receptor gene (SORL1) gene, which had long been hypothesized to be involved in AD pathogenesis [18]. Notably, 51A is considered overexpressed in vitro models and in the AD brain [19]. Massone et al reported that 17A would impair GABA signaling, enhance Aβ secretion, and increase the Aβ-42/Aβ-40 ratio [15]. Moreover, 17A is upregulated in AD subjects compared with control group, indicating that it could directly or indirectly be take part in the mechanism of AD [20]. Brain cytoplasmic 200 RNA (BC200) is a translational adjustor that targets eukaryotic initiation factor 4A, and making for the maintenance of long-term synaptic plasticity [21]. Based on the previous paper, BC200 RNA is upregulated in the AD brain and at least one study reported BC200 downregulation [22]. This conflict between multi-studies may be due to discrepancy in brain regions and varying disease severity, but aberrant BC200 expression in AD is a possibility [13].
Minor comments:

1. There is an anacronym "CAD" in the abstract. What does this stand for?

RE: We are very sorry for our incorrect writing. We have changed the “CAD” to “AD” in the abstract section.

2. The Methods section describes the preparation of samples from the AD cohort but not the control cohort. Were the methods different?

RE: Revised as suggested in the new manuscript. Special thanks to you for your comments.

3. The Methods section describes 2 different sites of primers used for BC200. Perhaps there is a mistake there? Was one meant to be 51A?

RE: Revised as suggested in the new manuscript. We are very sorry for our careless.

4. Table 1 should include the percentages of subjects in each category.

RE: We have added the percentages of subjects in the new Table 1.

Sylvain Lehmann (Reviewer 2):

In this manuscript, the authors report small differences in the level of some LncRNA from patients with or without Alzheimer's diseases. The work is very preliminary and need additional information and corrections. The results are also over interpreted.
Specific comments

1. The correlation analyses needs to be performed also on the all populations

RE: We have added the correlation analyses according to your comments in the new Table 2 and 3.

2. charaterisation of patients with MRI/CSF biomarkers will add to the work

RE: Revised as suggested in the Methods section.

3. forward and reverse are the same for BC200.

RE: We are very sorry for our incorrect writing. We have changed the reverse in the new manuscript.

4. the AUCs clearly indicate that the biomarkers are not usable or meaningful for AD diagnosis, it is therefore misleading that the indicate a specificity of 88%...

RE: As reviewer suggested that we have deleted the “specificity of 88%” in the revised manuscript.

5. statistical differences between ROC curve are needed

RE: We have added the statistical differences between ROC curve using medcalc software in the new Fig. 3.
6. validation of the result on an additional independent set of samples is absolutely needed.

RE: We agree with reviewer’s view that validation of the result on an additional independent set of samples is needed. Because above observation was made with a small population (n=160), we should further studied the diagnostic value of LncRNA BACE1 in a big population of patients. However, in this study, we unable to validate BACE1 in a big sample due to the condition limitation, we should spend more time to enroll more subjects in future. Moreover, in future, we should conduct more cooperation with some specialized organization to carried out multi-center clinical research rather than single-center. Special thanks to you for your comments.