Reviewer’s report

Title: Genome-wide association study identified ATP6V1H locus influencing cerebrospinal fluid BACE activity

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Reviewer: Cintia Barros Santos-Reboucas

Reviewer’s report:

In this manuscript, Hu and colleagues investigated the influence of a series of single nucleotide polymorphisms in cerebrospinal fluid (CSF) β-site APP cleaving enzyme (BACE) activity among Alzheimer's disease patients (AD), mild cognitive impairment (MCI) patients and healthy controls. Data used in the manuscript were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database. Although the authors did not find any significant differences in CSF BACE activity among the three groups, one main SNP (rs1481950) in the ATP6V1H gene was found by the first time to influence CSF BACE activity in both AD and MCI patients. These results suggest that ATP6V1H gene may play a role in the neurodegenerative process. Even though the study has some limitations recognized by the authors, including the limited sample size, it could add new insight into the current literature, opening new avenues for replication in larger datasets.

Major points:

- The manuscript lacks a detailed functional description about the ATP6V1H gene. Authors should discuss the role of ATP6V1H and its encoded protein, known as a component of vacuolar ATPase (V-ATPase) in the neurodegenerative process related to mild cognitive impairment and Alzheimer's disease. Such protein is ubiquitously expressed in brain and acts as a multisubunit enzyme that mediates acidification of intracellular organelles, which is necessary for multiple processes including protein sorting, zymogen activation, receptor-mediated endocytosis, and synaptic vesicle proton gradient generation.

- Also, it is essential that the authors postulate some possible functional consequences for the intronic SNP rs1481950. How this variant could impair ATP6V1H function? Is it located in a conserved region involved in splicing of any of their six transcripts? What about qualitative and quantitative expression studies between homozygous individuals harboring the minor allele (GG) against wild type homozygous individuals (TT)? ATP6V1H is expressed on blood, which makes this evaluation possible.

- Authors could discuss the frequency of rs1481950 among different ethnic populations.

- Line 89: According to the text, all patients with AD in ADNI cohort have an early onset profile. However, patients' ages in table 1 range from 56.4 to 89.1 years, which includes late onset AD.
So, authors should describe better the profile of AD patients (sporadic X familial; early onset X late onset) for comparative purposes with other studies.

- Line 187: Authors could add the nomenclature NM_015941.3:c.871-5558G>T for describing the polymorphism rs1481950 for the first time.

- Lines 231-236: Although previous studies found that AD subjects have increased cerebrospinal fluid (CSF) BACE enzymatic activity compared with the controls, the authors did not find any significant differences in CSF BACE activity among the three studied groups, which is in line with two previous studies including an ADNI cohort study. Authors could describe better which kind of inclusion criteria and methodological differences could have contributed for their unexpected findings.

Minor Points:

- Abstract, line 62: "…which indicated that ATP6V1H gene may play a certain role in the pathogenesis of…" - please remove "certain".

- Introduction, line 79: please add the information about ADNI (Alzheimer's Disease Neuroimaging Initiative database, adni.loni.usc.edu).

- Lines 109-110: "Samples were obtained from 382 ADNI subjects, enrolled at 56 participating centers using previously reported methods for CSF measurements" - please provide here some references for CSF measurements.

- Lines 113-141: Methodology description of BACE enzymatic activity assay is extensively detailed. Authors could reduce this section, adding references for previous reports.

- Lines 223-230: This paragraph is unnecessary. The chromosomal location of the seven genes containing the suggestive SNPs could be described in a supplemental table, with their respective functions.

- Line 232-233: "This aspect of our research is in line with two previous studies including an ADNI cohort study" - please add the references.

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.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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