Reviewer’s report

Title: Peripheral Vascular Responses to Acetylcholine as a Predictive Tool for Response to Cholinesterase Inhibitors in Alzheimer's Disease

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Reviewer: Hermona Soreq

Reviewer's report:

This is an interesting and carefully conducted study aimed at pursuing differences in the peripheral response to acetylcholine administration of diagnosed Alzheimer's disease patients as predictors of their response to anticholinesterase therapeutics. The authors measured several response parameters and correctly comment that a significant part of such patients fail to benefit from anticholinesterase therapy and propose their test as a classification approach to predict the prospects of such treatment in diagnosed Alzheimer's disease patients. Their findings demonstrate a certain level of predictability which is the strength point of this article.

However, this study also involves major weaknesses. Specifically, it completely ignores ample readily available information which can shed light on both the outcome of their test as well as on the prospects for successful treatment with Alzheimer's disease therapeutics. Such information includes sex- and age-related differences in circulation cholinesterase levels and the neurogenetics knowhow on single nucleotide polymorphisms (SNPs) in both the acetyl- and butyrylcholinesterase AChE and BChE genes. Additionally, many recent articles refer to the risk of cognitive decline in aged individuals under treatments with anti-cholinergic therapeutics, but that aspect as well has not been considered in this study.

Notably, genomic differences were studied by many and were shown to change the cholinergic status (namely, the total capacity in one's circulation to hydrolyze acetylcholine) and reaction to cholinesterase inhibitors. Those genetic changes may alter the coding sequence and/or the 3'-untranslated regions (3'-UTR) of the relevant transcripts where microRNAs targeting these transcripts control the levels of produced enzymes. Additionally, the relevant information may have distinct predictability for the two relevant genes.

For example, BChE is the main cholinesterase in the circulation but a relatively minor contributor to the brain's cholinesterase content; therefore, inherited or acquired changes in this gene's product may be more relevant to the peripheral test than to the disease treatment. In comparison, a SNP in the 3'-UTR of the AChE gene impairs interaction with this transcript of the primate-specific microRNA-608 and was recently shown to lead to 40% excess of brain AChE activities. Such change is likely to affect the responsiveness to anticholinesterases, more than the reaction to the peripheral test; and the genotyping approach is readily available for experimentation, for this and other genomic changes- but the presence of this and other SNPs was not even considered in this article.
In conclusion, the following comments call for revision and additional tests:

1. Separate men and women in the analyses;
2. Determine AChE and BChE activities in serum and/or plasma samples of the tested patients at both time points;
3. Report anti-cholinergic therapeutics taken by the tested volunteers for other reasons (e.g. allergy or incontinence) which would likely change their reaction to the administered acetylcholine.
4. Cite Alkalay et al. in CAR 2012 for the association between blood cholinesterase activities and brain amyloid load in Alzheimer's disease patients and Hanin et al., HMG 2014 for SNP-related differences in brain AChE activities.
5. Refer to genotype differences in cholinergic transcripts as likely to be causally involved in both the reaction to the peripheral test and the treatment.
6. Add AChE, BChE to the list of abbreviations.

**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.

No

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

No

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If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

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