Author’s response to reviews

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

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Editor Comments:

Please ensure the role of each individual author is described in the author contributions section (using initials rather than full names)

The role of each individual author is now described in the author contributions section using initials.

Eugenie Nepovimova (Reviewer 1):

We are very pleased with the comments from Reviewer 1 acknowledging that the study was a pilot study and recommending publication of the article.

Hermona Soreq (Reviewer 2):

Reviewer 2 raised some very interesting and pertinent points that would certainly contribute to a more detailed interpretation of our findings and point to more specific underlying mechanisms that could affect the responses we found and the response to treatment. We believe that these additions are not within the scope of this pilot study and would be need to be explored as part of a larger follow-up study, which we have in preparation. Our specific responses to the comments are detailed below.

1. Separate men and women in the analyses;

We agree that examining gender differences in the response to treatment is a valuable addition. However, due to the small number of patients in this pilot study, we believe that separating the analysis for men and women would significantly reduce the statistical power of our analysis and potentially
introduce a statistical error. We did however adjust for gender in the ANOVA model (which did not change the outcome) and have included this in results on page 10. “The change in peak acetylcholine response, peak sodium nitroprusside response and acetylcholine 50% decay from baseline to 6-month follow-up was significantly different between non-responders and responders after adjusting for baseline values, BMI and gender (P<0.002, P<0.01, and P<0.001, respectively)”. Importantly, we state in the Discussion that “...an important point to note is that the purpose of our study was not to determine what factors (e.g. BMI, blood pressure, gender) influence outcome to treatment, but rather to see whether we could find a biomarker indicative of response to treatment based on examining the peripheral vascular response to iontophoresis of acetylcholine”.

2. Determine AChE and BChE activities in serum and/or plasma samples of the tested patients at both time points;

We agree that in a more detailed study, analysis of AChE and BChE activities in blood samples would enhance the scientific validity. However, as this was a pilot study, for which we did not have consent for research blood tests, we are unable to provide these data. As mentioned for the effect of gender differences the main purpose of our study was to see whether we could find a biomarker indicative of response to treatment rather than the underlying mechanisms.

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.

None of our patients were taking anti-cholinergic therapeutics for other reasons. We have stated in the Methods on page 4 “Patients were excluded if they had previously been treated with cholinesterase inhibitors, had medical conditions known to interfere with acetylcholine metabolism or were likely to react adversely to skin stimulation”.

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.

We have now cited papers by Alkalay et al. 2012 and Hanin et al 2014.

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.

We have added information in the Discussion on page 12 (regarding genotype differences in cholinergic transcripts that could be causally involved in both the reaction to the peripheral test and the treatment.

We are aware that a peripheral test can only be a proxy for central activity and a weakness of our pilot study is that it did not allow for detailed examination of the mechanism which might affect response, including potentially important genomic findings in single nucleotide polymorphisms (SNPs) in both the acetyl- and butyrylcholinesterase AChE and BChE genes which may have a strong influence on the responses found in our test (Hanin et al 2014) but possibly also in treatment. It is highly likely that no single variable will prove to be a fully predictive measure of response but detailed mechanistic evaluation can be explored as part of a larger follow-up study in preparation.
“We appreciate that additional factors that were not assessed in this study could change the cholinergic status and alter the capacity to hydrolyse acetylcholine and thus the responses found in our test (Alkalay et al. 2012, Hanin et al. 2014) but possibly also in treatment. We are also aware that a peripheral test can only be a proxy for central activity and a weakness of our pilot study is that it did not allow for detailed examination of the mechanism which might affect response including potentially important genomic findings in single nucleotide polymorphisms (SNPs) in both the acetyl- and butyrylcholinesterase (AChE and BChE) genes which may have a strong influence on the responses found in our test (Hanin et al. 2014) but possibly also in treatment. However, the strength of our findings is that they are generated by a minimally-invasive test which can be performed fairly quickly in a clinic setting and potentially provide a rapid pragmatic guide to clinicians and patients about likelihood of response to cholinesterase inhibitors. It is highly likely that no single variable will prove to be a fully predictive measure of response but detailed mechanistic evaluation can be explored as part of a larger follow-up study in preparation.”

6. Add AChE, BChE to the list of abbreviations.

AChE and BChE have been added to the list of abbreviation.