Reviewer's report

**Title:** Effects of Early- and Mid-Life Stress on DNA Methylation of Genes Associated with Subclinical Cardiovascular Disease and Cognitive Impairment: A Systematic Review

**Version:** 0  **Date:** 03 Oct 2018

**Reviewer:** Jennifer Smith

**Reviewer's report:**

This manuscript is a systematic review of the relationship between early and mid-life stress and DNA methylation. Specifically, the review focuses on genes that have been previously been shown to have associations between DNA methylation patterns and either cardiovascular disease or cognition. Stress exposures are defined relatively broadly, ranging from infectious disease status (HIV viral load) to chronic disease (sleep apnea) to social exposures (SES), among others.

**Background**

1. The authors discuss the overlap in risk factors for CVD and CI, and they suggest that there may be shared genetic/epigenetic risk factors for the two. However, there is no information about the state of the genetic literature in this area. So, for example, have there been specific genes that have been linked to risk of both CVD and CI? If so, which biological pathways are these genes associated with?

**Methods**

1. P4-5: May be best to have the complete list of search terms in the supplemental material.

2. P5, line 7: The authors excluded paternal and prenatal stress from the scope of the stress exposures. Are maternal stressors included, and if so, why? For example, maternal separation is an exposure that is included, but this seems to also be an individual stress exposure (rather than simply a maternal exposure). Please clarify.

3. It is not explicitly stated why CI as a symptom of a specific disease (ex. AD) is excluded. Also, please discuss whether pathological disease processes (like AD) could have affected results in some cohorts (those with participants >age 60). Finally, was clinical CVD excluded as well (such as those where the outcome was MI)?
4. P6, line 4: the authors indicate that gene(s) with the greatest association are included in the table. For those in which the complete list of genes is not provided, please indicate this in the table with a footnote.

Results

1. How do the "methylation method" categories in Table 1 correspond to the venn diagram in figure S2? S2 is somewhat confusing, because it's not clear which types of studies fall into each of the four categories provided. For example, is whole genome methylation profiling getting global measures of DNA methylation, getting CpG site measures through epigenome-wide arrays, or both, or neither? Another point of confusion is bisulfite conversion, which is used in bisulfite sequencing, but is also a step in array processing. Then, search for differentially methylated regions can be done with multiple methods including array and sequencing technologies.

2. In Table 2, the Peter 2016 study used the 450K beadchip. However, under "regions analyzed", the authors discuss DML. In actuality, the "regions analyzed for methylation" is the entire genome. The DML are the regions that were identified in the analysis.

3. The title for Figure 2 is "Genes analyzed in more than one study …". Is this correct, or do the authors mean "Genes identified in more than one study …"?

Discussion

1. How much is known about different stress-related biological processes that may be associated with different types of stressors (for example, infectious disease vs. sleep apnea), and can the authors speculate on how these different exposures may have influenced results (or what could be done to examine convergence in effects)?

2. It is not important only to control for things like race and sex, but also to look for differences in stress-methylation and methylation-CVD/CI across strata.
Minor comments

1. P1, line 3: "serve to partially explain"

2. P2, line 29: "CVD-related"

3. P2, line 26; P6, line 7: Formatting issue with references

4. P4, line 3: "so as to"

5. In some cases, the authors refer to the analysis as a 'rapid review' and others as a 'systematic review' … what's the difference between the two?

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.

Not relevant to this manuscript

Quality of written English
Please indicate the quality of language in the manuscript:

Acceptable
Declaration of competing interests

Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal