Author’s response to reviews

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

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Version: 1 Date: 20 Dec 2018

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Dr. Zhen-Yu Zhang
Associate Editor
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November 14, 2018

Subject: Revision and resubmission of manuscript MGTC-D-18-00303
Dear Dr. Zhang,

Thank you for your letter and the opportunity to revise our paper on ‘Effects of Early- and Mid-Life Stress on DNA Methylation of Genes Associated with Subclinical Cardiovascular Disease and Cognitive Impairment: A Systematic Review.’ The suggestions offered by the reviewers have been immensely helpful, and we also appreciate your insightful comments on revising the Methods and Discussion sections, as well as additional critical aspects of the paper.

I have included the reviewer comments immediately after this letter and responded to them individually, indicating exactly how we addressed each concern or problem and describing the changes we have made. Since we added a reference in response to one of the reviewer’s suggestions, we changed the superscript numbers following the studies included in Tables 1 and 2, which are highlighted in red. The revisions have been approved by all four authors and I will be serving as the corresponding author. The changes are highlighted with red in the paper as you requested, and the revised manuscript is attached to this email message.

We hope the revised manuscript will be found acceptable for publication in BMC Medical Genetics but are happy to consider further revisions, and we thank you for your continued interest in our research.

Sincerely,

Elena Vidrascu, MSc

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Reviewer 1

Background

Question 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?

Response: A literature search was conducted to discover any genes that have been linked to risk of both CVD and CI, and only one 2004 review was found (nothing more recent). The review concluded that only the Apolipoprotein E (ApoE) gene was consistently found among studies to be associated with Alzheimer’s disease. This gene is involved in the metabolic pathway and helps to breakdown fats. This information has been added to the manuscript Background section to further support our argument that there is a lack of this important information.

Reference:


Location of Response: P3, L6-10

Methods

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

Response: We agree with this suggestion as we thought it looked too crowded, so we made the necessary change and added the terms to the Supplementary Material.
Question 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.

Response: Thank you for catching this typo. We had meant to write “parental,” instead of “paternal.” This change was made, as well as clarifying the reason for including maternal separation as a stressor.

Location of Response: P4, L18-19; P18, L5

Question 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)?

Response: We wanted to capture preclinical risk factors for CI and CVD, so clinical CVD was excluded. We didn’t want CI to be specific to a particular disease (e.g. dementia in AD), but rather for it to be applicable to the general population. We did have studies where participants were old enough to have pathological disease present, and it is possible that these processes could’ve affected results; however, only one 2004 review identified one common gene implicated in both CVD and neurological disorders (AD), and this gene wasn’t identified in any of our included studies. We do identify this as problematic, and future studies should consider controlling for pathological disease processes if they wish to explore CI as an independent outcome. We added this suggestion to our Discussion section.

Reference:

Location of Response: P4, L8-9; P5, L2-3; P17, L22-24
Question 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.

Response: Thank you for this recommendation. A footnote with this information is included for both Tables 1 and 2.

Location of Response: P8; P11

Results

Question 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.

Response: We originally had thought that grouping together the methylation methods, according to Figure 1 in Reference 73, would help support our argument that there is a lack of consistency between studies, including the methylation methods used. However, we understand that Figure S2 further complicates things and so we have decided to entirely remove it and any reference to it in the manuscript. We believe that Table 1 alone sufficiently details the methylation methods used in each study.

Location of Response: P17, L12

Question 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.

Thank you for
clarifying this distinction. We made this change in Table 2 for both the Peter 2016 and Levine 2016 studies, and in Table 1 for the Nanayakkara 2008 study.

Location of Response: P8; P11

Question 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 …"?

Response: We had meant to say the latter, and we appreciate you catching this error.

Location of Response: P13, L16

Discussion

Question 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)?

Response: This is a very interesting question. A particular stressor may induce illness in one person but not in another, partly due to the stress response being highly personalized, and partly due to factors like cognitive resources and coping ability. It appears that most studies and reviews analyze different psychological stressors and their effects on biological processes (e.g. immune function), depending on type of stressor and its duration. There is a lack of research on other types of stressors, such as illness (e.g. sleep apnea). This illness might be perceived as a stressor by one person, but not by another. For this reason, we suggest in our Discussion that stress assessments be utilized in order to identify types of stress (psychological vs. biological), duration of stress, etc., and then if studies also control for illnesses, it might be easier to parse out whether methylation changes are entirely due to the illness itself, or also partly in result of the psychological stress response.
Question 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.

Response: We absolutely agree with this statement, and have addressed this suggestion in the Discussion section.

Location of Response: P16, L19-21

Minor Comments

Question 1: P1, line 3: "serve to partially explain" (Response: P1, L3)

Question 2: P2, line 29: "CVD-related" (Response: P2, L19)

Question 3: P2, line 26; P6, line 7: Formatting issue with references (Response: P2, L26; P5, L19)

Question 4: P4, line 3: "so as to" (Response: P4, L5)

Response (Questions 1-4): Thank you for catching these typos. We have carefully proofread the revised manuscript.

Question 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?

Response: We had initially meant for this review to be “rapid,” implying that it was going to be completed within a short time frame (<3 months), but due to the broad range of inclusion criteria, yet narrow scope of the research question, we decided to keep it as a systematic review. This change was made in the manuscript.
Reference:


Location of Response: P3, L20

Reviewer 2

Question 1: Those reviewed articles are relevant. Genes selected by each study were described and results were evaluated. However, there was a lack of elevation of the appropriate use of statistics in each of the reviewed studies. As there are different methods of analysis of epigenetic changes, each of them would need different statistical analysis. The authors should also review on this aspect so that the credibility of the results came from a particular study could be better appreciated. These comments could append to the 2 tables.

Response: We understand that a meta-analysis of the statistical methods utilized in each study could identify the significance of the overall effect of stress on methylated genes, but there was considerable variability among studies so we decided against this. The authors agree that reviewing the statistical methods of each study, based on methylation method used, is important, but we decided that this would be outside the scope of the review and it would be more pertinent to delineate the methods of analysis of epigenetic changes and comment on this.

Location of Response: P18, L2-4