Author's response to reviews

Title: Modification by hemochromatosis gene polymorphisms of the association between traffic-related air pollution and cognition in older men: a prospective cohort study

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Author's response to reviews: see over
Editors, Environmental Health

To the editors:

Thank you for the opportunity to re-submit our manuscript, titled “Modification by hemochromatosis gene polymorphisms of the association between traffic-related air pollution and cognition in older men: a prospective cohort study,” for publication in Environmental Health as a research article. We appreciate and have addressed all of the reviewers’ comments and have revised the manuscript accordingly. A point by point response to the reviewers’ comments can be found appended to this letter. Changes to the manuscript, with the exception of formatting changes, are denoted using the “track changes” function of Microsoft Word.

All coauthors have read the manuscript, agree that the work is ready for submission to a journal, and accept responsibility for the manuscript’s contents. This manuscript is an original work. The contents of this manuscript have not been previously published, are not under consideration for publication elsewhere, and will not be submitted or published elsewhere while under consideration by Environmental Health. Participation of human subjects did not occur until after informed consent was obtained. None of the authors have any competing interests.

Thank you for your consideration.

Regards,

Melinda C. Power, Corresponding Author
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Reviewer: Erin Semmens

Reviewer's report:

This paper describes a hypothesis-driven investigation of the potential modification by HFE polymorphisms of traffic-related air pollution effects on cognitive performance in older men. The writing is clear, and the study is an interesting extension of published work. Studies of air pollution effects on central nervous system function are relatively new, and this work provides some insight into the mechanisms underlying the associations observed in several large epidemiologic investigations. Below are issues to consider and address.

Thank you.

Major Compulsory Revisions

None

Minor Essential Revisions

1) Background, paragraph 2: Briefly summarizing the other work that has been done examining the effects of air pollution exposure on the brain would provide a stronger public health motivation for conducting the work described.

Thank you for this suggestion. We have added a summary of the current epidemiologic work on the association between air pollution exposure and cognition to the end of paragraph 2 in the Background.

2) Methods, Cognitive testing: Although described in the previously reported analysis, please discuss the clinical significance of an MMSE score # 25.

As suggested, we have added text to the Methods, Cognitive testing section, immediately following the statement that MMSE were dichotomized at 25. This text reads: “…(while a score of $\leq 24$ is commonly used as a cutoff for dementia screening in research settings, very few of our participants met this criterion).”

3) Methods, Statistical Methods, paragraph 2, sentence 2: “Using separate models for each analysis…” Please clarify that “each analysis” refers to each SNP and to each cognitive outcome (i.e., the cognitive z-score and the MMSE
score).

Based on the comments of the current reviewer and Beate Ritz, we have re-written or re-organized substantial portions of the statistical methods section for improved clarity (and re-ordered parts of the results section to follow the same logic). Part of this re-write addresses this comment: “We began by evaluating the main effect of each of the two HFE SNPs, H63D and C282Y, on the odds of having a low MMSE score and on total cognition in four separate models. We then added multiplicative interaction terms between the pertinent HFE SNP and black carbon to each of these four models to assess the potential for effect modification of the black carbon-cognition association by each HFE SNP.”

4) Methods, Statistical Methods, paragraph 2: It would be helpful to report what statistical test was performed and what threshold was used to define significance of interactions between BC and HFE SNPs.

As suggested, we have added this information to the revised statistical methods section: “We used Wald tests of the multiplicative interaction term and a p-value threshold of 0.05 to assess whether there was statistically significant support for effect modification of the black carbon-cognition relationship by HFE SNP in each model.”

5) Results, paragraph 2: Although the authors touch on this in the last sentence of the paragraph, it would be helpful to more explicitly report results for statistical tests of interaction, which should then be referenced again in the Discussion. The fact that significant associations were observed between BC and both the cognitive z-score and low MMSE score in those lacking an HFE C282Y variant is intriguing. However, while it is important not to over-emphasize a p-value, the fact that the interaction was not statistically significant in any case deserves mentioning.

Thank you for this suggestion. We now realize that the use of the term “suggest” or “suggestive” does not adequately reflect to the reader the reality that the p-values for interaction are not statistically significant, despite the interesting finding of no association in variant carriers. We have changed our language throughout the abstract, results, and discussion to be more explicit, so as to not oversell our findings.

Discretionary Revisions

1) Please provide a more detailed discussion of how the exposure metric of interest was selected. If baseline cognitive tests occurred sometime between 1996 and 2007, then exposure estimated for the year preceding the baseline assessment could conceivably stretch over more than 10 years. How did concentrations change over time? If they changed substantially or differentially, how might this have affected results? Were any other metrics used to define
long-term exposure to BC (e.g. choosing mean exposure during a fixed calendar year for everyone)?

While the reviewer raises a good point, we chose not to add a more elaborate discussion of this decision here, as this is a straightforward extension of previous work and we did not re-consider our original analytic decisions when assessing effect modification by HFE. That said, we recognize that time trends could possibly bias our results and when completing the original analysis, we considered several other metrics, including using a one-year average for a single calendar year of exposure for each participant. Ultimately, we found that the choice of metric did not materially change our conclusions. In addition, as it is difficult to see how HFE status could be systematically related to the timing of cognitive testing/associated exposure estimates, we would not expect this choice to impact the current analyses.

2) Discussion, paragraph 1: Would the authors provide some context for what “strong adverse associations” mean? For example, the effect of BC exposure on cognition in those lacking the C282Y variant was similar to that observed with an x year increase in age or y years additional education.

Thank you for this suggestion. We have added the text to this sentence to provide context in terms of an x year increase in age, as follows: “While participants who lacked an HFE C282Y variant exhibited strong adverse associations between BC and total cognition, a magnitude of effect that is equivalent to approximately 2.1 years of age in our data, we did not observe an adverse association for participants with at least one variant genotype.”

3) If performed, please mention results of analyses examining how HFE SNPs might modify the effects of long-term BC exposure on cognitive trajectories over time in those who had multiple cognitive assessments.

We did not do analyses for the association between BC exposure and cognitive trajectories. We hope to do this analysis, as well as to evaluate modification by HFE, upon receipt of further cognitive data (as we currently have an average of 2 cognitive assessments per person, we did not feel that we had sufficient data to adequately model cognitive trajectory, given expected practice effects from test 1 to test 2 in addition to expected decline over time).

Reviewer: Beate Ritz

Reviewer's report:

This is an interesting paper exploring possible gene-environment interactions building upon an earlier report published by the same authors in which they found that black carbon (BC) estimates of traffic pollution from a spatiotemporal land use regression model for the greater Boston area in 1995 were associated with cognitive function in elderly men from the Normative Aging Study (NAS).
The candidate gene they identified for this purpose is the hemochromatosis gene (HFE) which had previously been found to modify short term air pollution effects on cardiovascular outcomes in the NAS. The authors provide appropriate biologic justification for selecting this gene that acts in inflammatory and oxidative stress pathways hypothesized to also influence cognitive decline in the elderly. The paper is well written and the results clearly presented and discussed.

Thank you.

Major revisions:

1) The main issue with this paper is whether one is inclined to agree with the authors interpretation that their results suggest and interaction between the variant polymorphism in HFE and BC exposure since the 95% CI of the variant estimate for both the total cognitive score and the low scoring on the MMSE overlap largely with the wild type estimates and CIs and the p-values for interaction are 0.2 and 0.11 which would not be considered formally statistically significant. Thus the authors should at least comment on their use of the term ‘suggest’. The lack of a negative association in variant carriers is however intriguing and worthwhile to be noted.

Thank you for this suggestion. As mentioned in response to the previous reviewer, we now realize that the use of the term “suggest” or “suggestive” does not adequately reflect to the reader the reality that the p-values for interaction are not statistically significant, despite the interesting finding no association in variant carriers and an adverse association in non-carriers for HFE C282Y. We have changed our language throughout to be more explicit, so as to not oversell our findings.

2) The authors describe using “all” cognitive tests conducted for each person in multiple waves of testing, i.e. baseline and follow-up exams. They seem to have standardized results to the baseline test score, but it is unclear whether or how they used multiple tests per person. Is it correct that the beta they present represents a rate of cognitive decline? This should be stated more clearly. Also, how would this work if there was only one test result available i.e. the one from baseline?

Our analyses use multiple tests and multiple assessments per person, but are estimating the impact of black carbon exposure on level of cognitive test performance, not change in test scores over time. Essentially, although we are using data from multiple assessments, the interpretation of our model betas is the same as if we had only used a single cognitive assessment per individual. We made the choice to use multiple assessments to improve our power to detect an effect, as our sample size is relatively small and the expected effect size (both the main effect of black carbon, as reported in the original manuscript, as well as the associations by HFE status, reported in this manuscript) are also expected to be small.
We made the conscious decision not to look at the association between black carbon and cognitive trajectory. With, on average, a little over 2 assessments per individual, we did not feel that we could adequately model the expected cognitive trajectory, which includes improvement from test 1 to test 2 due to practice effects as well as cognitive decline with the passage of time. We hope to do the analysis of the association between BC and cognitive decline upon receipt of additional cognitive test data.

Based on this and other reviewer comments, we realized that our statistical methods section needed to be edited for clarity, and we have revised accordingly. We also revised the results section to better reflect the new organization of the methods section.

3) In their original paper the authors explicitly addressed potential confounding by lead since traffic-related exposures were a primary source of lead exposure in the era of leaded gasoline. Even though this did seem to change main effects for total cognition in the previous paper it by more than 10%, thus, why did they choose to not adjust for lead exposure in the GxE analyses presented here?

We agree with the reviewer that the sensitivity analysis from the original paper where we adjust for lead exposure should be repeated in this analysis. In the original analyses, adjustment for lead (through use of a combination of imputed and measured tibia bone lead concentrations) impacted our “total cognition” analysis estimates slightly but did not change the MMSE analysis. We considered this a sensitivity analysis in the original paper and in the current manuscript because of the requirement that we use imputed estimates of lead exposure, rather than measured estimates, for approximately 45% of the sample. We now describe and report sensitivity analyses adjusting for the combined imputed and measured tibia bone lead levels in both the results and discussion sections. Our results were materially unchanged after adjustment for lead burden.

4) Are the betas and ORs presented in tables 2 and 3 from two different models, i.e. are they indeed then marginal rather than main effects? The p-value for interaction - I presume - is from a model with both an interaction term and both main effects in the same model. I don’t understand the sentence in the ‘stat method section’ that states “Using separate models for each analysis, we then evaluated the main effects of each HFE SNP on cognition and the potential for effect modification of the black carbon cognition association by HFE SNPs using multiplicative interaction terms.” What is meant by separate models? Stratified analyses? Also they state that “As the current dataset excludes participants from the previously reported analyses [2] who lacked valid HFE genotyping, we also reproduced the previously reported main effect models in this reduced dataset.” How can these be ‘main effects’ previously reported when there were no interactions tested, i.e. shouldn’t this be marginal effects then?

The betas and ORs from Tables 2 and 3 were derived using 4 separate models. In Table 2, we represent the effect estimate from two different linear mixed models with the outcome of total cognition – one for C282Y and one for H63D – with the basic form of $Y = B0 + B1(SNP) +$
B2(ln(BC)) + B3(SNP*ln(BC)). The p-value for interaction is the Wald p-value for B3, the beta for “wild type” is B2, and the beta for variant is B2+B3. Similarly, Table 3 represents the effect estimate from two different logistic regression models for the outcome of having a low MMSE score.

In response to this and previous comments, we have substantially revised the “Statistical Methods” section of the manuscript for clarity and now follow the order of the revised statistical methods section when reporting results. We also re-labeled “Beta” in Table 2 to read “Expected difference in total cognitive z-score.” We hope that these revisions eliminate any confusion over how to interpret the findings reported in Tables 2 and 3.

Reviewer: C. Arden Pope

Reviewer's report:

Major comments:

1. This paper used data from over 600 participants of the VA normative Aging Study. Traffic related air pollution exposure was estimated by estimating black carbon (BC) using a land use regression model. In a previous paper they reported that traffic related exposure was associated with measures of cognition. In this paper the evaluated whether or not this association was modified by hemochromatosis gene polymorphisms. They found that the adverse BC-cognition association appeared to be modified by the HFE C282Y but not the HFE H63D genotype. These somewhat suggestive results are interesting and potentially important.

Thank you.

2. The paper is succinctly written and the analysis is conducted by a well-respected research team with relevant experience.

Thank you.

3. The paper is well-motivated and is a natural extension of the previously reported work that found adverse BC-cognition associations and effect modification for cardiovascular disease outcomes and polymorphisms in the hemochromatosis gene. The paper also provides some discussion of proposed biological mechanisms.

Thank you.

4. In general, the weakest aspect of the paper is, although well motivated, the
actual results are not highly compelling. Given that the statistical inference of an adverse association was at best marginally significant, given that there are multiple testing issues, and given that the results are not fully consistent with presumed priors, the results are mostly only suggestive. In fairness, the authors do not severely oversell the results and the statement that this finding and the proposed biological mechanism require confirmation is certainly true.

We agree that these results should not be oversold. As mentioned in response to the other two reviewers, we have edited throughout to be more explicit about the fact that the p-value for interaction was at best marginally significant, although still potentially of interest, given that our model indicates no association in one group and an adverse in the second.

5. In the background a brief discussion of the background and importance of HFE polymorphisms (including relevance outside of the air pollution literature, with key cites) would be helpful.

Thank you for this suggestion. We have now added a paragraph to the background section providing broader context of the importance and relevance of HFE polymorphisms on human health.

“The hemochromatosis (HFE) gene regulates iron homeostasis. Two common missense polymorphisms of this gene, HFE C282Y and H63D are associated with the disease state of hemochromatosis, an autosomal recessive genetic disease that causes an increase in absorption of ingested iron. Although the penetrance is low, this can, over time, lead to iron overload, manifesting in higher rates of diabetes, heart disease, and liver disease [12, 13]. Variation in these HFE polymorphisms may also appear to impact body burden of other metals, particularly divalent cations like manganese, lead and cadmium [14-18].”

Minor comments:
1. Page 4, 1st paragraph, line 2. “there are many reasons why we may . . . “ Suggest “there are many reasons to be interested. . . “

Thank you for this suggestion. We have made this edit.


Thank you for this suggestion. We have made this edit.

Upon re-reading this sentence, we realized that we omitted a reference to Baja 2010. Therefore, we now cite three studies and have replaced the word “several” with “previous.” The sentence now reads: “Previous studies have reported that HFE polymorphisms modify the association between air pollution exposure and cardiovascular outcomes or risk factor [19-21]. “

4. Page 12, line 7. Redundant use of the word “directly”.

Thank you for catching this. We have deleted the first “directly” and the sentence now reads: “Oxidative stress and inflammation may contribute to the development of cognitive impairment directly or through inducement of cardiovascular problems.”