Reviewer's report

Title: Ambient pollutants, polymorphisms associated with microRNA processing and adhesion molecules: the Normative Aging Study

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Reviewer: Ralph J Delfino

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Review of Wilker et al. Environ Health “Ambient pollutants, polymorphisms associated with microRNA processing and adhesion molecules: the Normative Aging Study”

General Comments
This interesting study addresses a novel area of research on effect modification of air pollutant exposure-response relations by polymorphisms in genes that process microRNAs. Authors found that a SNP in an miRNA processing gene (rs1062923) modifies associations of sICAM-1 and sVCAM-1 levels with 7-day average PM2.5 and SO42-.

Major concerns:
There was no discussion in the limitations section about the limited number of repeated measures, including subjects with only one measurement (N unknown). What were the results dropping subjects wit no repeated measures? In other words, if all subjects had only one measurement, then this would be a cross-sectional study with its potential biases. This is not the case of course, but the separation of the few measurements by months or years adds to the potential for temporal confounding, which should be more vigorously controlled for in the analysis. By chance, different subjects with high VCAM and ICAM could have come for an evaluation during periods of high air pollution (a sort of temporal / cross-sectional bias).

The paper gives the appearance of a selective choice of exposure averaging time even though there were statements suggesting that averaging times were to be explicitly tested in the analysis. There was insufficient direct evidence that the choice of a 7-day average was more strongly associated with outcomes than shorter times.

A major influence of smoking on adhesion molecules suggests that the analysis should be tested excluding smokers (who would be differently affected by ambient air pollution anyway).

Specific Comments:
Abstract:
Reconsider using the term endothelial dysfunction as equivalent to ICAM and
VCAM changes with which it is associated. Endothelial dysfunction denotes an imbalance between vasodilation and vasoconstriction.

Introduction:
P 4, line 112: These references describe not only cardiovascular function (blood pressure) but also blood biomarkers, which are not measures of function. Are there any other similar studies with evidence supporting longer averaging times?

Methods:
P 7: How far was the stationary site from subject residences (range and average)?
P 8: You say “Our a priori hypothesis was to examine 7 day moving averages.” However, in the Introduction and abstract a hypothesis referred to showing differences by shorter vs. longer averaging times. This requires multiple averaging times in the analysis to answer. I suggest a typical lag 0 and 2- or 3-day average. Later you mention 5 and 9-day averages were examined and were more weakly associated, suggesting that odd averaging times were tested including 1, 3, 5, 7 and 9 days. All of these results should be shown

P 9, top, analysis: Since subjects came into the clinic during different seasons and years on one or a few occasions, then this should be controlled for in the analysis. By chance, different subjects with high VCAM and ICAM could have come for an evaluation during periods of high air pollution (a sort of cross-sectional bias).

Results:
P 11: a bit over half way down the sentence (An IQR …” needs rewording.
P 11 end 1st paragraph: It would informative to show the results for BC and for other averaging times as discussed above since it is unlikely you could have predicted which one was the strongest.
P 12: although it is unlikely that BC would interact with the SNPs being itself NS, it is possible. Since we cannot see BC results, it’s hard to evaluate this possibility.

Discussion:
P 13, 1st para: You say “In this repeated measures study” but in fact, some unknown number of subjects had only one measurement. To really claim that this is the design you would have to drop those subjects. What happens to the results when you do that?
P 13, 1st para: You say “Results from our sensitivity analyses may suggest that even longer averaging times are relevant …” but this analysis is not shown.
P 13, 1st para: You say “carriers of the variant had lower levels of the markers of endothelial function” but you did not measure endothelial function.

P 13, 1st para: plural SNPs was used but results for only one SNP were shown.

P 13, 2nd para: association of what with BC?

P 13, 2nd para: VCAM and ICAMM are not “vascular function.”

P 14, 1st para top: it’s power plants.

P 14, 2nd para: not “vascular function.”

P 14, 2nd para: Results were not presented to evaluate the statement: “rs1062923-PM2.5 crossproduct predicting adhesion molecule level was consistently the strongest of all interactions tested”

P 15, 2nd para: “day-today variability” could not be assessed since the subject measurements were widely separated in time. Later there is a statement that “Varying susceptibilities of study subjects and differences in time windows studied may have also contributed to different outcomes.” I’m not sure, but I think this is where you are admitting to the problem of temporal/cross-sectional bias from measurements widely separated in time and taken at non-discrete intervals in different subjects.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests