HFE, air pollution, and HRV
ARIC study manuscript proposal

ARIC Manuscript Proposal #1310

1.a. Full Title: Role of air pollution measured as particulate matter concentration on the association between HFE gene mutations and heart rate variability.

b. Abbreviated Title (Length 26 characters):
HFE, air pollution, and HRV

2. Writing Group:
Writing group members:
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I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. _S.K.A._ [please confirm with your initials electronically or in writing]

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3. Timeline: Following approval of this proposal and subsequent data release, this work will lead to a manuscript within nine months.

4. Rationale:
Exposures to air pollution measured as the concentrations of ambient particulate matter (PM) and other criteria pollutants (O₃; SO₂; NO₂; CO) have been shown to decrease pulmonary function [1], and to increase hospital admissions [3] [4], cardiovascular and

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all-cause mortality [2] [5, 6]. PM is bound loosely to positively charged, divalent metallic cations such as iron (Fe++) [7].

Ambient PM [8-10] and specific pollutants like SO₂ [11, 12], O₃ [13], and metals [14] are also associated with higher levels of inflammatory or oxidative markers. Autonomic system regulated changes in the body’s oxidative state have been implicated as one of the mechanisms by which such pollutants alter cardiac function [15].

Among the various implicated cations, Fe++ remains much studied as a causative agent for increased cardiovascular mortality. A recent study by Park et al found that HFE gene mutations modify the association between exposure to PM < 2.5 µm in aerodynamic diameter and heart rate variability (HRV) [16] such that among individuals with the wild type HFE gene, there is an inverse PM–HRV association, whereas no such association exists among those with HFE mutations.

Well-characterized, prevalent HFE gene mutations (e.g. C282Y and H63D) have been used in epidemiological studies as markers of hereditary hemochromatosis (HH), an autosomal recessive genetic disorder of excess Fe++ absorption, accumulation, and overload. These mutations appear to be associated with higher serum Fe++ and ferritin concentrations, but lower absorption and serum concentrations of other toxic divalent metallic cations (Pb++; Cd++; Cu++; Co++; Mn++) [17]. For example, they are associated with lower Pb++ concentrations in humans [18]. It is important to note that although these mutations are expressed as increases in Fe++ load (i.e. transferrin and ferritin levels [19]), they don’t account for all the Fe++ overload. Other unidentified genes and mechanisms also contribute to Fe++ overload among individuals with HH. [20]

In addition to differences in Fe++ absorption and metabolism, individuals with and without HH also absorb and metabolize other airborne divalent cations in different ways. These differences may cause variations in localized or systemic oxidative stress, serum concentrations of other divalent cations, and autonomic or cardiovascular health [16]. HRV is a well-known, sensitive marker of cardiovascular changes following acute and chronic PM exposure.

Decreased HRV, a marker of autonomic imbalance, is associated with cardiovascular mortality [21]. HRV is decreased in individuals exposed to higher PM or other criteria air pollutant concentrations [9, 10, 13, 22-36]. Similar observations have been made among persons with coronary artery disease, lung disease, [37, 38] [12, 34] [39] and diabetes [40-43]. Age [32], gender, obesity [44], cardiovascular disease (CVD) [30, 45], hypertension [31] and diabetes have been identified as modifiers of the PM-HRV association.

Interestingly, the study by Park et al shows no PM-related decrease in HRV among individuals with HFE gene mutations [16]. The authors implicated HFE mutation-related absorption of Fe++ from the lungs as a possible explanation for this finding. However, the study’s finding is not consistent with the hypothesis that Fe++ overload leads to CVD-related morbidity and mortality, since decreased HRV is also associated with increased CVD and all-cause mortality [46, 47]. Moreover, results from studies examining the hypothesis that Fe++ overload increases coronary heart disease (CHD) and CVD mortality have been inconsistent. An initial cohort study found increased CVD risk with higher ferritin levels [48], whereas findings from subsequent studies have been mixed. Importantly, a meta-analysis including twelve prospective studies did not find any
association between Fe** overload and CHD [49]. A recent randomized clinical trial found no benefit of graded phlebotomy in reducing CHD and related mortality among patients with peripheral vascular disease. However, the therapy was found beneficial in a subset of young participants. Differences in the metabolism of divalent cations related to HFE mutation may help explain the inconsistent results. Failure to reject the null hypothesis among participants with HFE mutation in Park et al also may have been related to low power and/or unavailability of homozygous HFE mutations. Having said that, the finding that HFE mutations and Fe** overload are also associated with type 2 diabetes [50] [51]—a condition jointly associated with decreased HRV [53-55]—makes simple explanations unlikely.

Park et al studied a small population (n=518) of mostly elderly Whites from a single community using a cross-sectional design and relying on mean PM concentration data collected one day before participant examinations at one site. The ARIC cohort provides an opportunity for replicating analyses conducted by Park and extending them to include analyses of allele dose in a distinct, bi-ethnic and much larger population-based, multi-community sample. Measures of PM and HRV (from ten-second, two- and six-minute recordings) are available from four clinical visits over 12 years.

To summarize, air pollution is associated with decreased HRV. Divalent metallic cations like Fe** may affect the association by altering the local and systemic oxidative/inflammatory milieu. HFE gene mutations are used in epidemiological studies as markers of increased Fe** absorption/storage and lower concentrations of other potentially toxic divalent ions like Pb++. The Fe** overload-CVD hypothesis does not have a solid footing despite extensive research. The protective effect of HFE gene mutations (by maintaining autonomic balance; reducing local pulmonary inflammation; increasing Fe** absorption; decreasing concentrations of other divalent metallic cations) may dilute the effect of increased Fe** stores on associated heart disease. We wish to study the putative modification of the PM-HRV association by HFE gene mutations while controlling for demographic characteristics and co-morbid conditions. The ARIC study will support examination of the above interaction using longitudinal environmental and electrocardiographic data from four visits in four communities. It will also allow us to study the consistency of the association across strata defined by race, age, gender and CVD risk factors. Moreover, its large sample size will allow us to study the effects of allele dose.

5. Main Hypothesis/Study Questions:
(1) The inverse association between heart rate variability measures and ambient PM10 concentrations (in µg/m³) measured at one-, two- and three-day lags before ARIC visits is modified by HFE gene mutation
(2) The effect of HFE gene mutation
   a) Depends on allele dose
   b) Is consistent across age, center/race and gender groups
   c) Is consistent across HRV
      i) recording duration (10-sec ECG [V1-4]; 2- and 6-min heart rate recording [V2 & V4])
      ii) time domain measure (RR; SDNN; RMSSD)
      iii) frequency domain measure (HF; LF; HF/LF ratio)
   d) Is robust to simultaneous adjustment for
      i) other CVD risk factors
      ii) use of iron supplementation
6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodological limitations or challenges if present).

Exclusions: Participants who did not consent to genotyping will be excluded (use of DNA data distributed by the Coordinating Center with confirmation by using the variable RES_DNA = “No use/storage DNA” in the file ICTDER02).

Study Design and Analyses:
We will employ a longitudinal design using data from eligible ARIC participants at all visits to examine whether the HRV-PM association is modified by HFE genotype. Skewed dependent variables will be transformed, as needed, to approximate Gaussian distributions. We will use SAS PROC MIXED to conduct a random-effects, multilevel analysis. Center (Level 3) will be modeled as a fixed effect. Within center, PM will be modeled initially as a random effect (intercept; slope). Within participant (Level 2), visit/time (Level 1) also will be modeled as a random effect. Nuisance effects of the correlation among serial measurements within participants will be controlled using a repeated statement.

Variables:

From Visit 1-4:
ARIC community; 24 hr average of PM\textsubscript{10} for one, two and three days prior to the examination; Weather variables: relative humidity and temperature; HRV measures from 10 sec ECG: RR, SDNN and RMSSD; CVD risk factors: BMI, diabetes, glucose, hypertension, blood pressure, smoking, LDL cholesterol, heart disease, renal disease, and COPD; Medication use: beta blockers, anti-arrhythmics, and iron supplementation.

ARIC Visit 1:
Demographics: age, race and gender; HFE gene mutations

ARIC Visits 2 and 4:
Time domain measures from 2 and 6 min heart rate recording: RR, SDNN and RMSSD; Frequency domain measures from 2 and 6 min heart rate recording (HF; LF; HF/LF ratio).

Statistical analysis

7.a. Will the data be used for non-CVD analysis in this manuscript? __X__ Yes ___ No

b. If Yes, is the author aware that the file ICTDER02 must be used to exclude persons with a value RES_OTH = “CVD Research” for non-DNA analysis, and for DNA analysis RES_DNA = “CVD Research” would be used? __X__ Yes ___ No
(This file ICTDER02 has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript? __X__ Yes ___ No
8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER02 must be used to exclude those with value RES_DNA = “No use/storage DNA”?  

**X** Yes  ____ No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at:  http://www.cscc.unc.edu/ARIC/search.php

**X** Yes  __  No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)? The authors were invited to serve on this manuscript and have consented to collaborate.

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?  

**X** Yes  ____ No

11.b. If yes, is the proposal

A. primarily the result of an ancillary study (list number* _AS 2003.10, AS 1998.01_ )

B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* _Yes_____

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

12. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.

References:


