

ARIC Manuscript Proposal # 2924

PC Reviewed: 02/13/17 **Status:** _____ **Priority:** 2
SC Reviewed: _____ **Status:** _____ **Priority:** _____

1.a. Full Title: Particulate Matter Air Pollution and Leukocyte Traits

b. Abbreviated Title (Length 26 characters): PM and DNAm

2. Writing Group: WHI-EMPC & ARIC Epigenetics Working Groups
Writing group members: Jan Bressler, Myriam Fornage, Weihua Guan, Ellen Demerath, Jim Pankow, and Kari North

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. __RG__ **[please confirm with your initials electronically or in writing]**

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3. Timeline: Primary analyses & draft manuscript to be completed by late 2017 or early 2018

4. Rationale:

Ambient particulate matter (PM) air pollution is a modifiable exposure that has been consistently associated with cardiovascular disease (CVD) morbidity and mortality.¹ Despite the ubiquity of air pollution exposure and the continued population burden of PM, the molecular associations that underlie PM-associated cardiovascular health effects have not been completely described.

DNA methylation (DNAm), a heritable but dynamic epigenetic modification that can influence gene expression without altering the genome, may be central to mediating pathways by which environmental factors modify CVD risk.² Although several studies have attempted to quantify causal mediation by DNAm³⁻⁶, assumptions critical to the study of mediation have not been rigorously evaluated. For example, causal mediation analyses may not be valid if a variable affected by the exposure under study (e.g. PM concentration) is also a confounder of the mediator-outcome (e.g. DNAm-CVD) association⁷⁻¹⁰.

Leukocyte composition may be such a variable, either when measured via cytometry as part of a complete blood count / differential, or in its absence, when estimated by constraining the sum of the CD8+ T cell, CD4+ T cell, natural killer cell, B cell, monocyte, and granulocyte proportions in whole blood to 100%, then fitting a regression model to DNAm data.¹¹ Indeed, the ability of leukocyte composition to confound epigenome-wide association studies is usually controlled by including measured or estimated leukocyte proportions as co-variables in DNAm-outcome association models.

Epidemiologic studies also suggest that exposure to PM increases leukocyte count. However, the evidence is inconsistent and largely based on small¹²⁻¹⁹, cross-sectional¹²⁻¹⁶ studies conducted in special populations¹⁹⁻²⁷ or panels²⁸⁻³⁰ with limited generalizability. Moreover, such studies often fail to comprehensively evaluate effects of PM on leukocyte composition^{19-25, 28-31}.

The proposed study will therefore evaluate associations between PM, leukocyte count, and composition by leveraging repeated measures and estimates of them in large, multi-racial, geographically diverse U.S. populations enrolled in the Atherosclerosis Risk in Communities (ARIC) study and the Women's Health Initiative (WHI). Study results will guide future approaches to causal mediation analyses of PM, DNAm, and CVD.

5. Main Hypothesis/Study Questions:

To examine the associations between leukocyte count and composition with concentrations of ambient particulate matter ≤ 2.5 , ≤ 10 , and between 2.5 and 10 micrometers in diameter (PM_{2.5}, PM₁₀, and PM_{2.5-10}).

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 methodologic limitations or challenges if present).

Overview. The general approach is to estimate in ARIC 1) PM-leukocyte count associations at V1-V5; 2) PM-measured leukocyte composition associations at V1, V2, and V5; and 3) PM-

estimated leukocyte composition associations in a subset of participants at V2, V3. These analyses will rely on air pollution data generated as part of the “*Modification of PM-Mediated Arrhythmogenesis in Populations*” ancillary study (MOPMAP; R01-ES017794; PI – Whitsel). Analogous estimates of 1-3 will be generated in WHI and combined with those from ARIC in fixed-effects, inverse variance-weighted meta-analyses.

Study Population. Leukocyte count is available in approximately 15,546 participants. Measured leukocyte composition is available in approximately 11,457 participants. Estimated composition is available in a subset of African American participants at V2/3 ($n \approx 2,750$) and will soon be available in a subset of European American participants ($n \approx 1,180$). Corresponding samples sizes in WHI are approximately 160,116; 7,399; and 2,200 for leukocyte count, measured composition, and estimated composition, respectively.

Exclusions. Leukocytosis, leukopenia, and common conditions associated with established abnormalities of leukocyte count and/or composition, including hematological malignancy or use of an oral/parenteral glucocorticosteroid, granulocyte/macrophage colony stimulating factor, lithium, or antibiotic (as a proxy for infection).

Outcomes. Leukocyte count and leukocyte compositions (measured; estimated). Measured leukocyte composition was determined by cytometry as part of a complete blood count / differential, i.e. proportions of lymphocytes, monocytes, basophils, neutrophils, and eosinophils. Likewise, estimated leukocyte composition, defined by CD8+ T cell, CD4+ T cell, natural killer cell, B cell, monocyte, and granulocyte proportions, will be estimated using Houseman methods¹¹. Proportions will be isometrically log-ratio transformed³³ in preparation for multivariate, compositional data analysis^{34,35}.

Main Exposures. Geocoded participant address-specific 2-, 7-, 28-, and 365- day mean concentrations of ambient PM_{2.5}, PM₁₀, and PM_{2.5-10} regulated under the Clean Air Act by the U.S. Environmental Protection Agency (EPA) according to its National Ambient Air Quality Standards (NAAQS). Concentrations at the time of blood draw were estimated using national-scale, log-normal kriging and EPA Air Quality System monitoring data.³⁶ Data on PM_{2.5} was not widely available until 1999, so before that year, its concentrations were instead estimated using generalized additive mixed models, the log-transformed ratio of PM_{2.5} to predicted PM₁₀, and geographic information system (GIS)-based predictors.³⁷ PM_{2.5-10} for each averaging period was calculated as the difference of PM₁₀ and PM_{2.5}.

Covariates. Demographic covariates (age; center), relevant meteorological covariates, seasonality, and potential confounders of interest (erythrocyte and platelet counts, smoking status, alcohol use, body mass index, physical activity, individual-level education, and neighborhood socioeconomic status).

STATISTICAL ANALYSIS

For each PM size fraction and exposure averaging period, covariate-adjusted, multi-level, linear mixed-effects longitudinal models will leverage repeated measures to estimate associations between PM, leukocyte count, and composition (measured; estimated). There will be a random

intercept and slope for time at the participant level and for PM at the ARIC center level. Similar models will also be used to estimate cross-sectional associations using a random intercept and slope for the ARIC center level. For analyses of leukocyte composition, multi-variate methods of Aitchison³³ and others³⁴ will be implemented. Complementary analyses will be conducted in WHI, and fixed-effects inverse variance-weighted meta-analyses will be used to combine the study-specific test statistics. Sensitivity of results to additional adjustment for clinical variables and to substitution of imputed personal for ambient exposures will be examined, as previously suggested.³⁸⁻⁴⁰

CONCLUSIONS

In this study, we will estimate associations between ambient particulate matter air pollution and leukocyte traits, the nature of which may ultimately provide insight into the biological consequences of exposure and guide future approaches to causal mediation analyses of PM, DNAm, and CVD.

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

b. If Yes, is the author aware that the file ICTDER03 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? Yes No
(This file ICTDER 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? 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 ICTDER03 must be used to exclude those with value RES_DNA = "No use/storage DNA"? 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.csc.c.unc.edu/ARIC/search.php>

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)? NA

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? Yes No

11.b. If yes, is the proposal

- A. primarily the result of an ancillary study (list number* 2009.08)**
 B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* _____)

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

12a. 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.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is **your responsibility to upload manuscripts to PubMed Central** whenever the journal does not and be in compliance with this policy. Four files about the public access policy from <http://publicaccess.nih.gov/> are posted in <http://www.csc.unc.edu/aric/index.php>, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to PubMed central.

13. Per Data Use Agreement Addendum, approved manuscripts using CMS data shall be submitted by the Coordinating Center to CMS for informational purposes prior to publication. Approved manuscripts should be sent to Pingping Wu at CC, at pingping_wu@unc.edu. I will be using CMS data in my manuscript Yes No.

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