1.a. Full Title
Associations among Ambient Air Pollution, Genetic Risk Factors and Age-related Macular Degeneration in the Atherosclerosis Risk in Communities (ARIC) Study.

b. Abbreviated Title (Length 26 characters): Air pollution and AMD.

2. Writing Group:
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3. Timeline: Analyses are planned to be completed between October and December 2019

4. Rationale:

**Age-related macular degeneration**

Age-related macular degeneration (AMD) is a leading cause of severe visual impairment among older adults worldwide, with the disease burden expected to increase exponentially over the next few decades given the current trends of population aging.\(^1\)\(^2\) Vision loss in AMD largely stems from disruption of the retinal pigmentary epithelium (RPE) in advanced disease, marked by extensive RPE loss with subsequent deterioration of the...
overlying neurosensory retina (geographic atrophy; advanced non-exudative AMD) and/or breakdown of the blood-retina barrier leading to development of choroidal neovascularization (advanced exudative AMD). Available therapies serve to slow progression from early disease to exudative AMD, but do not reverse existing retinal damage or slow progression to geographic atrophy. Moreover, AMD pathogenesis is complex and remains unclear, highlighting the need for additional epidemiologic research focused on clarifying the underlying molecular mechanisms and identifying potential modifiable risk factors.

**Oxidative Stress and AMD**

Oxidative stress that incites inflammation and disrupts lipid metabolism within the RPE may be the common insult leading to AMD pathogenesis. The RPE serves various supportive functions critical to the visual cycle and the health of the retina, including absorption of scattered light; uptake of glucose, carotenoids and lipoproteins; phagocytosis of lipid-rich mature photoreceptor outer segments; and maintenance of the blood-retina barrier. However, these roles and the close proximity to phototransduction processes renders the RPE highly susceptible to direct free radical injury from light-induced formation of reactive oxygen species and lipid peroxides. Damaged RPE cells then accumulate metabolic waste (drusen), which together with free radicals, activate the complement system and cellular stress response pathways, as well as recruit inflammatory mediators such as macrophages and T-cells. Immune complex deposition then exacerbates structural injury and intensifies the inflammatory cascade, culminating in RPE/retinal atrophy and aberrant angiogenesis, each distinctive characteristics of AMD progression. In accordance with the theorized mechanism, oxidative stress is known to increase with both aging and cigarette smoking, the two strongest risk factors for AMD. Moreover, increased dietary intake of lutein and zeaxanthin – xanthophyll pigments that are concentrated in the macula and help filter high-energy blue light as well as reduce scavenge free radicals – has been consistently associated with lower risk of advanced AMD. High-dose antioxidant supplementation has further been shown to decrease risk of progression from intermediate to advanced AMD.

**Ambient Air Pollution as a Novel Risk Factor for AMD**

Cigarette smoking is the strongest known modifiable risk factor for AMD, and tobacco smoke and traffic-derived air pollution share many similar components – including particulate matter and oxidizing gases such as ozone. Chronic ambient air pollution (AAP) exposure could plausibly induce a parainflammatory state that promotes oxidative stress and development of AMD via several pathways. AAP may incite a local inflammatory response within the lungs, with subsequent systemic spillover of cytokines and other proinflammatory mediators. In addition, there may be direct translocation of fine particles (i.e. particulate matter ≤2.5μm in diameter) and diffusion of gases across the alveolar membranes into the systemic circulation, which in turn may lead to increased oxidative stress and vascular endothelial dysfunction. Finally, direct contact of particulate matter with the ocular surface and diffusion of gaseous pollutants across the cornea may contribute to heightened intraocular inflammation.

In accordance with these hypotheses, AAP has been associated with elevated biomarkers of systemic inflammation and oxidative stress. AAP exposure has also been shown to influence retinal vascular caliber, which in turn has been correlated with systemic inflammation, endothelial dysfunction, as well as AMD. Moreover, AAP exposure has been associated with various inflammatory ocular surface conditions including dry eye syndrome, blepharitis, and allergic conjunctivitis. Collectively, these data suggest that AAP may be a novel, potentially modifiable risk factor for AMD.

**Genetic Risk Factors and AMD**

Genetic factors associated with increased oxidative stress and inflammation have been shown to increase AMD risk. In particular, two single-nucleotide polymorphisms strongly associated with AMD – complement factor H (CFH) rs1061170 (Y402H, T1277C) and age-related maculopathy susceptibility 2 (ARMS2) rs10490924 (A69S) – may increase early AMD risk by 1.5-fold. CFH encodes for a soluble protein involved in downregulation of the alternative complement cascade. CFH rs1051170 polymorphism alters the binding properties of the gene product,
leading to aberrant complement activation, increased inflammation and elevated oxidative stress.\textsuperscript{47-51} On the other hand, \textit{ARMS2} rs10490924 may impair the physiological activation of the alternative complement pathway, resulting in parainflammation, impaired phagocytosis of cellular waste, and formation of drusen.\textsuperscript{52-54}

To date, there have been no studies examining the associations among these polymorphisms, AAP, and AMD. However, smoking and other environmental factors leading to increased oxidative stress may interact synergistically with \textit{CFH} rs1061170 and \textit{ARMS2} rs10490924 to influence AMD risk.\textsuperscript{55} In the Blue Mountains Eye Study, current smokers carrying at least one risk allele of \textit{CFH} rs1061170 were found to be at significant higher risk for advanced AMD than current smokers who were non-carriers as well as non-smokers who were carriers.\textsuperscript{56} The Nurses’ Health Study and the Health Professionals Follow-up Study, as well as the Rotterdam Study, also revealed additive interactions between \textit{CFH} rs1061170 and smoking on AMD.\textsuperscript{57,58} In contrast, several large case-control studies, the Age-Related Eye Disease Study, and the Beaver Dam eye study found independent but not multiplicative interactive effects of smoking and \textit{CFH} rs1061170 on AMD.\textsuperscript{55,59-62} Of note, data from the Nurses’ Health Study and the Health Professionals Follow-up Study also showed no multiplicative interaction between \textit{CFH} rs1061170 and smoking.\textsuperscript{57} Similarly, several large case-control studies and a meta-analysis found evidence of both multiplicative and additive interactions between smoking and \textit{ARMS2} rs10490924 on AMD,\textsuperscript{54,63-65} though there are also reports of null associations.\textsuperscript{62,66} Given the similar composition of cigarette smoke and AAP, \textit{CFH} rs1061170 and \textit{ARMS2} rs10490924 may modify the effects of AAP on AMD.

Genetic variants in apolipoprotein E (\textit{ApoE}) may also influence both AMD risk and the relationships among AAP and AMD. However, the pattern of association is less clear, given that the \textit{ε4} allele has been shown to lower AMD risk in meta-analyses,\textsuperscript{67} but at the same time may heighten sensitivity to the neurocognitive effects of ambient air pollution.\textsuperscript{68} Furthermore, \textit{ApoE ε2} has been shown to increase odds of advanced exudative AMD among smokers.\textsuperscript{69} Since the retina is considered an extension of the central nervous system, these data suggest that the associations among \textit{ApoE} genotype, ambient air pollution, and AMD also warrant further investigation.

5. **Main Hypothesis/Study Questions:**

   **Main Study Question 1:**
   Is there an association between ambient air pollution (AAP) exposure and prevalent early/intermediate AMD?

   **Main Study Question Hypothesis:**
   \textit{High AAP exposure will be associated with increased odds of early/intermediate AMD.}

   **Main Study Question 2:**
   Is the association between AAP and early/intermediate AMD modified by \textit{CFH} rs1061170, ARMS2 rs10490924, and \textit{ApoE} genotypes?

   **Main Study Question 2 Hypothesis:**
   \textit{AAP will be associated with higher odds of early/intermediate AMD among carriers of \textit{CFH} rs1061170 C, ARMS2 rs10490924 T and \textit{ApoE ε2 alleles than among non-carriers.}

   **Additional Study Questions:**
   Does smoking status and race modify the association between AAP and early/intermediate AMD?
   Does dietary lutein and zeaxanthin intake modify the association between AAP and early/intermediate AMD?
   Is retinal vascular caliber a pathway variable in the association between AAP and early/intermediate AMD?
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).

Study Sample

The ARIC Study is a population-based prospective cohort designed to investigate the natural history of atherosclerosis and its associations with cardiovascular disease outcomes, as well as the potential influence of race, sex, and healthcare disparities. At visit 1 (1987–1989), a total of 15,792 participants between the ages of 45 and 64 were recruited via probability sampling of four geographic regions in the United States: Forsyth County, North Carolina; Jackson, Mississippi; Minneapolis, Minnesota; and Washington County, Maryland. At visit 1, visit 2 (1990–1992), and visit 3 (1993–1995), participants underwent physical examination and completed surveys inquiring about sociodemographic factors, lifestyle choices, and medical history. Grorable fundus photographs were obtained from 11,209 of the 12,887 participants who returned for visit 3 (no retinal photographs, n=160; ungradable photographs n=1,480). In addition, approximately 796 eligible individuals declined to provide consent for research unrelated to cardiovascular disease outcomes. Thus, the initial study sample will consist of an estimated 10,413 participants.

Disease endpoints

Prevalent AMD was determined via fundus photographs taken at visit 3 (1993-1995), using a non-mydriatic automatically focusing camera (Canon CR-45UAF; Canon U.S.A., Inc.; Itasca, IL) and without pharmacologic dilation. Patients were asked to sit in a darkened room for 5 minutes, after which the camera was centered on the region including the optic disc and the fovea of a randomly chosen eye. Nonstereoscopic 45-degree color film retinal image thus obtained were then evaluated by a masked grader at the University of Wisconsin Fundus Photograph Reading Center for the presence of soft drusen >63µm in diameter, changes in RPE pigmentation, geographic atrophy, and choroidal neovascularization. Given the low number of advanced AMD cases (n=15, 0.14%), the primary endpoint variable will be prevalent early/intermediate AMD, defined as presence of soft drusen >63µm or RPE depigmentation, in the absence of geographic atrophy and choroidal neovascularization (estimated n=596, 5.7%).

Assessment of retinal vascular caliber

For assessment of retinal vascular caliber, the fundus photographs were converted to digital images and then magnified. A masked grader then marked all vessel boundaries of retinal arterioles and venules lying within a zone of 0.5-1 disc diameter from the optic disc. For arterioles >80µm in diameter at the border of this zone, the first two branches distal to the border were also evaluated. The vessel diameters were then measured by computer, and summarized as central retinal artery equivalents (CRAE) for arterioles and central retinal vein equivalents (CRVE) for venules. The arteriole-to-venule ratio was also calculated as CRAE/CRVE.

Assessment of Ambient Air Pollution Exposure

Participant residential addresses since ARIC inception have been accurately geocoded. Participant address-specific monthly mean concentrations of PM_{2.5} and PM_{10} between 1988 and 1995 were then spatiotemporally estimated using available US Environmental Protection Agency Air Quality System (AQS) data, generalized additive mixed models, and land-use regression. For gaseous pollutants (O_{3}; NO_{x}; NO_{2}; SO_{2}; CO), participant address-specific daily mean concentrations (maximum, 8-hour, rolling) for the same time period were estimated using AQS data and national-scale, log-normal, ordinary kriging. The pollutant-, duration-, and model-specific estimates will be averaged over five years up to (and including) dates of fundus photography. Corresponding estimates of relevant, participant address-specific covariates (i.e. meteorological factors and neighborhood socioeconomic status) were also computed.
Genetic data
Genotyping of single nucleotide polymorphisms (SNPs) in ARIC was completed using the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, Inc.; Santa Clara, CA). The ARMS2 rs10490924 (A69S) was genotyped directly as part of the Affymetrix chip. Subsequent imputation, using both HapMap and the 1000 Genomes reference panels as appropriate for Caucasians and African Americans, yielded data on CFH rs1061170 (Y402H). ApoE genotype was determined via restriction isotyping using the TaqMan Assay (Applied BioSystems, Inc.; Foster City, CA). Minor allele frequency and Hardy-Weinberg equilibrium for ARMS2 rs10490924 and ApoE genotypes were met. The imputation quality for CFH rs1061170 was high in both the Caucasian and African-American datasets (scores >0.8).

Assessment of tobacco smoke exposure
At visit 1 (1987-1989), participants completed surveys inquiring about sociodemographic factors, lifestyle choices, and medical history. This included data on pack-years of smoking and smoking status. Smoking status was also updated at visit 2 (1990-1992) and visit 3 (1993-1995), but data on smoking intensity was not obtained at either visit. For this study, we will therefore use pack-years and smoking status at visit 1 as measures of tobacco smoke exposure.

Assessment of dietary xanthophyll intake
A 66-item food frequency questionnaire (FFQ) completed at visit 1 (1987-89), 6 years prior to fundus photography at visit 6, will be used to estimate dietary intake of lutein and zeaxanthin (mcg). This instrument was modified from a version developed by Willet et al., and its validity and reliability has been demonstrated. Dietary lutein and zeaxanthin intake will be adjusted for daily caloric intake using the multivariate nutrient density model. Participants with implausible total energy intake (women: <500 or >3600 kcal; men <600 or >4200 kcal) or with >10 missing values on the FFQ will be excluded. Dietary supplements of lutein and zeaxanthin were not available during the time of the study.

Pooled Analyses using data from the women's health initiative
Using data from a well-characterized national cohort of community-dwelling postmenopausal women (Women’s Health Initiative, study sample n=5,419), we found that particulate matter <10 microns in diameter (PM10) was associated with greater odds of prevalent AMD. Moreover, when stratified by smoking status, the associations of both PM10 and PM2.5 with AMD were strongest among never-smokers (Lin et al unpublished data, under preparation). AAP exposure, meteorological covariates, and neighborhood socioeconomic status were calculated using identical methods as in ARIC. AMD status could be similarly categorized by the presence of early/intermediate AMD based on fundus photographs graded at the University of Wisconsin Fundus Photograph Reading Center. Data on retinal vascular caliber is not available in the Women’s Health Initiative. Nonetheless, we plan to examine the associations among AAP exposure, AMD status, and smoking status in the ARIC study as well as in the combined population.

Proposed analysis
The distribution of participant characteristics and other risk factors according to AAP exposure (quintiles), AMD status (none vs. early/intermediate), and the presence of pathologic stigmata of early/intermediate AMD (i.e. soft drusen, changes in RPE pigmentation) will be examined using chi-square test, t-test and analysis of variance. Bivariate relationships among these variables will also be explored using Pearson product-moment correlation, point-biserial correlation, or chi-square test. Tables summarizing these descriptive statistics will be presented.

Multivariable logistic regression will be used to evaluate the association between quintiles of AAP exposure and AMD status. Crude, age-adjusted and multivariable-adjusted odds ratios, 95% confidence intervals, and p-values for trend analyses (using quintile medians) will be reported. Potential confounders will be identified using
previous studies or the change-in-estimate method (≥10% change in the OR). Identified confounders will be adjusted for in subsequent multivariable analyses. Effect modification by *CFH*, *ARMS2* and *ApoE* genotypes as well as smoking status (never, former, and current smoker), race (non-Caucasian vs. Caucasian) and lutein/zeaxanthin intake (tertiles) will be assessed via interpretation of the multiplicative interaction terms, synergy indices and stratified analyses. Specifically, we will investigate interaction by adding multiplicative interaction terms (e.g., AAP exposure * genetic risk variable) to the regression model. A p-value for interaction of <0.10 will be considered statistically significant.

To test whether retinal vascular caliber could be a pathway variable for the association between AAP exposure and AMD status, the relationships between AAP exposure and retinal vascular caliber, as well as between retinal vascular caliber and AMD status will first be examined. If both relationships are statistically significant, the association between AAP exposure and AMD status with and without adjustment for retinal vascular caliber will then be assessed. Attenuation of the association between AAP exposure and AMD by inclusion of retinal vascular caliber in the regression model would suggest that AAP exposure may influence AMD status via effects on retinal vascular caliber.

**Limitations and possible solutions**

The proposed study has several limitations. First, the use of prevalent AMD as an outcome precludes inference of causality; it is impossible to determine whether maculopathy was already present at baseline. However, since the 5-year incidence of early AMD is relatively low in adults <75 years of age, disease odds may reasonably approximate disease risk in ARIC.

Second, in this sample, prevalence of advanced AMD was low (0.14%), even among persons over the age of 65 (0.4%). Thus, analyses will be limited to using early/intermediate AMD as the outcome of interest. Moreover, only 12,887 of the 26,427 participants (82% of the survivors) recruited at baseline (1987-1989) returned for Visit 3 (1993-1995). Of these individuals, 1,317 had ungradable fundus photographs, and may have been at greater risk of AMD (i.e. older, more likely to have diabetes mellitus, and more likely to have evidence of CVD on magnetic resonance imaging). Taken together with the high rate of non-participation, conclusions drawn from this study may be susceptible to selection bias. Multivariable analyses will therefore be interpreted with and without adjustment for the propensity score, or the conditional probability of an outcome given a set of observed confounders. The propensity score will be constructed using covariates that differed in distribution by fundus photograph status (gradable vs. ungradable).

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 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?

        _X_ Yes  ____ No

        (This file ICTDER03 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  _X_ 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”?

        _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 most related manuscript proposals would involve other work on age-related macular degeneration and would involve Drs. Ronald and Barbara Klein, both of whom are co-authors on this work.

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

____X____ A. primarily the result of an ancillary study (list number* 2010.20)

____ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* 2006.15)

*ancillary studies are listed by number at http://www.cscc.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.cscc.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.

References


