ARIC Manuscript Proposal #2702

PC Reviewed: 2/9/16	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Vitamin D Status and the Incidence and Progression of Age-Related Macular Degeneration: the Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): **Vitamin D and Incident and Progressed AMD**

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. __AEM__ **[please confirm with your initials electronically or in writing]**

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3. Timeline:

Analyses are planned to be completed between 02/02/16 and 05/01/16

4. Rationale:

Age-related macular degeneration (AMD) is the leading cause of irreversible vision loss (1), affecting ~6.5% of Americans 40 years and older (2). Inflammation is implicated in the pathogenesis of AMD (3-5), and vitamin D is hypothesized to prevent development of AMD, at least in part, through its anti-inflammatory and immune modulating properties (6-8). Existing literature supports a protective association between prevalent AMD and vitamin D status, assessed with the blood biomarker 25-hydroxyvitamin D [25(OH)D] reflecting intake from foods, supplements as well as sunlight exposure (9-11), although not all studies support this observation (12-14). Only two previous studies were conducted in racially diverse samples (9, 13), limiting our understanding of this association in racial groups such as African Americans who have one of the highest burdens of vitamin D deficiency (15). Currently no studies have investigated associations between vitamin D status and the incidence or progression of AMD.

The current body of literature needs large studies involving well-defined cohorts where AMD prevalence and severity is based on retinal photographs graded using reliable, standardized protocols, and vitamin D status is assessed using serum 25(OH)D concentrations with documented quality control measures to ensure minimal laboratory variation across batches of samples sent for analysis. These methods will help to minimize measurement error in both the exposure and outcome to enhance validity of study results. The Atherosclerosis Risk in Communities (ARIC) Study is a well-characterized epidemiologic cohort that has graded retinal fundus photographs for AMD and serum 25(OH)D measures assessed using liquid chromatography, mass spectrometry (LC-MS/MS) with implemented quality control measures. Retinal photographs were taken in ARIC participants at visit 3 (1993-1995) (n=11,863) and in a subset of participants at visit 5 (2011-2013) (n=1,298), allowing for assessment of AMD incidence and progression over ~18 years. Serum 25(OH)D concentrations were assessed in participants at visit 2 (1990-1992).

*This study is part of the approved **ARIC Ancillary Study 2010.20** "Relationships between vitamin D and retinal diseases of aging" as well as Specific Aim 3 of the **NIH/NIA funded grant R01 AG041776**, the "Role of Vitamin D Status in Retinal Diseases in Aging." Dr. Millen is the Principal Investigator of this work.*

5. Main Hypotheses/Study Questions:

Main Study Questions

Question 1: Is there an association between serum 25(OH)D concentrations assessed at visit 2 (1990-1992) and the incidence or progression of AMD from visit 3 (1993-1995) to visit 5 (2011-2013)?

Hypothesis Q1: *We hypothesize that ARIC participants with higher 25(OH)D concentrations will have less incident or progressed AMD as compared to those with lower 25(OH)D concentrations.*

Additional Study Questions

- Does age, race, gender, or genotype modify this association between vitamin D status and early 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 design:
These analyses will be conducted among participants with available serum 25(OH)D concentrations at visit 2 (1990-1992) and gradable retinal fundus photographs taken at visit 3 (1993-1995) and visit 5 (2011-2013). Of 12,091 eligible participants attending visit 3, 11,863 had nonmydriatic retinal photographs taken of one randomly chosen eye with film using a photo centered between the disc and fovea. A subset of participants from visit 5 were recruited to have retinal photographs with the target of including ~1,000 participants without any dementia or mild cognitive impairment comprised of 1) a random sample of eligible participants attending ARIC Visit 5 and 2) surviving participants of the ARIC Brain Magnetic Resonance Imaging (MRI) Study (2004-06). And ~1,600 participants with low neurocognitive test scores suggesting dementia or mild cognitive impairment (MCI) made up the additional subjects. Digital photograph without mydriatics were used to obtain retinal photographs (fields 1 and 2 (16)) of both eyes of participants at visit 5 follow-up. Previous research in this group has shown film and digital grading of AMD to be comparable (17) and thus it would be expected that incidence and progression of disease can be assessed between retinal photographs taken at Visit 3 with film and Visit 5 with a digital camera.

It was estimated that ~n=2,346 of the 2,637 participants would have had gradable retinal photographs taken at Visit 3 based on previous data in ARIC (18). However, due to recruitment difficulties, retinal photographs were only taken in both eyes in a smaller than estimated subset of participants (n=1,298). Of these, participants were excluded if they had ungradable photographs at visit 3 or 5 (n=387) or late AMD at visit 3 (n=1), making them ineligible for progression**.**

Participants were further excluded if they were not Caucasian or African American (n=2), if they were missing 25(OH)D (n=62) or other pertinent covariates (n=5). These 5 participants were missing data on either age, race, smoking status or hypertension status. This leaves an analytic sample of 841 participants (714 Caucasians, 127 African Americans) for this analysis.

At study visits 1-3, participants answered questionnaires on their lifestyle habits and medical history (19). They also had a physical exam and a blood draw (20). Prior to the visit, participants were asked to fast for twelve hours and to bring with them any medications or supplements they were taking or had taken within the past two weeks (19).

Disease endpoints:
Our disease endpoint is the incidence or progression of age-related macular degeneration (AMD) from visit 3 to visit 5. Among our sample of 841 participants with gradable retinal data at visit 3 and 5, eyes from photos taken at both visits were graded using the Wisconsin Age-Related Maculopathy Grading System (21) and, masked to grading results from earlier visits. Then a side-by-side grading was conducted on those eyes that had change across visits (either progression or regression) to confirm the incidence or progression of AMD.

At visit 3, there were 12 cases of prevalent early AMD. Between visits, 80 participants developed incident, early AMD; 11 participants had no AMD at visit 3 but developed late AMD at visit 5; and 3 participants progressed from early to late AMD.

Serum 25-hydroxyvitamin D:
Serum obtained at ARIC Visit 2 (1990-92) was used to assess 25(OH)D with liquid chromatography/tandem mass spectrometry (22) at the University of Minnesota, under the direction of Myron Gross (Minneapolis, MN). To adjust for season of blood draw, serum 25(OH)D concentrations will be regressed (using a local polynomial smoother) on day of the year. Residuals from this regression will be added back to the sample 25(OH)D concentration mean. This regression will be done separately in whites and blacks and the residual-adjusted 25(OH)D concentrations will be used in all subsequent analyses.

Other pertinent covariates:
We will investigate the following covariates as potential confounders: age, race, gender, education, income, health insurance, smoking status, drinking status, ethanol intake, weight, waist circumference, waist to hip ratio, body mass index (BMI), measures of serum total cholesterol, serum high density lipoprotein, serum triglycerides, and use of hormone therapy (in females), statins or aspirin. Diastolic and systolic blood pressure as well as hypertension status will be examined as pathway variables.

Genetic data: Genetic data are available in ARIC on a number of high risk AMD genes including *CFH* and *ARMS2*. Genotyping of single nucleotide polymorphisms (SNPs) in ARIC was completed using the Affymetrix Genome-Wide Human SNP Array 6.0 (23). Data are available on two high risk SNPs (*CFH* Y402H [rs1061170] and *ARMS2* A69S [rs10490924]) shown to be associated with increased risk of early AMD (24). We will use this data to explore whether either variant confounds or modifies the association between vitamin D and AMD.

Proposed Analysis:
We will use logistic regression to relate the log odds of incident or progressed AMD, in those with adequate ($\geq$50 to 75 and $\geq$75) compared to deficient or inadequate vitamin D status ($<$50 nmol/L). We will also examine 25(OH)D as a continuous variable, estimating the odds of incident or progressed AMD for each 10 nmol/L difference of 25(OH)D. We will estimate a p for linear trend using continuous 25(OH)D concentrations. Confounding will be investigated by adding potential confounders to our model in a step-wise fashion. Potential confounders will be added to the model if they change the OR 10% or more. We will stratify the analyses by age, gender, race, and genotype using an interaction term in the logistic regression model. A p-value of $<$0.10 will be considered statistically significant. In addition to the main analysis to investigate the effect of vitamin D status on overall incidence or progression of AMD, we will explore the effect of vitamin D status on incidence (any, early, and advanced AMD) as well as progression of disease without the inclusion of incident data.

Another significant limitation is potential bias due to loss to follow-up of participants with retinal photographs from visit 3 to visit 5. We will compare characteristics of participants with ocular data at visit 3 to those at visit 5 to better understand the potential bias. We will also conduct a sensitivity analysis using inverse probability weighting to account for loss to follow-up, as

previously done in ARIC analyses (25), to determine risk estimates for associations between vitamin D and AMD incidence or progression representative of the overall population-based ARIC cohort.

Limitations and possible solutions:
A limitation of our data is the availability of retinal photographs in only one eye using film at visit 3 for classification of retinal eye disease. Therefore, there may be misclassification of endpoints ascertained at visit 3 as well as in the determination of incident and progressed disease. As the eye chosen to be photographed at visit 3 was done so randomly, we would expect non-differential misclassification of our endpoint which would bias our observed risk estimates toward the null. We have digital retinal photos in both eyes at visit 5 and will be able to assess the degree of misclassification of these retinal diseases in the ARIC cohort participants if only one of the two eyes was used for determination of disease. This information will be used to investigate the effect of misclassification on our risk estimates. However, we have previously shown film and digital grading of AMD to be comparable (17).

Another significant limitation is potential bias due to loss to follow-up of participants with retinal photographs from visit 3 to visit 5. We will conduct sensitivity analyses (as described above) to investigate potential bias due to this attrition. We acknowledge that the observed relationship between vitamin D and AMD could differ among persons with and without dementia or MCI, although a biologic rationale for this is not foreseen. We will also conduct sensitivity analyses examining associations between vitamin D and incident or progressed disease in those without dementia or MCI.

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 be those involving Pam Lutsey's work on vitamin D and cardiovascular disease. Other relevant proposals are those that focus on diabetic retinopathy and would involve Dr. Ronald Klein. Both Drs. Lutsey and Klein 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)*

*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.

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