1.a. **Full Title**: Vitamin D, Vitamin D Metabolic Pathway SNPs and Risk of Cardiovascular Diseases: the Atherosclerosis Risk in Communities (ARIC) Study

b. **Abbreviated Title (Length 26 characters)**: VitD SNPs & CVD

2. **Writing Group**: Writing group members: Mary R Rooney, Pamela L Lutsey, James S Pankow, Erin D Michos, Jared P Reis, Casey M Rebholz, Katie C Hootman

   Other interested investigators welcome

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

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**ARIC author** to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).  
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3. **Timeline**: Data analyses will begin immediately. Goal completion is Summer 2016.

4. **Rationale**: Worldwide, an estimated 1 billion people are vitamin D [as measured by 25(OH)D] deficient or insufficient (1). Suboptimal vitamin D concentration has been associated with higher risk of cardiovascular disease (CVD) (2-6). Low 25(OH)D tends to be more common among
those with darker skin pigmentation (1, 7). However, recent evidence suggests that the association of 25(OH)D with major outcomes may vary by race (7-12).

Vitamin D metabolism is complex and intricately regulated. Recently, numerous candidate SNPs have been identified on genes involved in the vitamin D metabolic pathway, i.e. vitamin D binding protein (VDBP), cubilin (CUBN), 1-α hydroxylase (CYP27B1), and vitamin D receptor (VDR) (13, 14). While 3 prior studies have found inconsistent results (15-17), it is possible that polymorphisms related to these protein-encoding metabolism genes may modify associations of low 25(OH)D with CVD risk.

In a 2012 JAMA publication, Levin et al examined whether vitamin D metabolic pathway SNPs modified the association between 25(OH)D and major clinical outcomes in Cardiovascular Health Study participants and replicated findings through meta-analysis with 3 other cohorts (15). Of 5 SNPs examined, 2 VDR gene SNPs (rs7968585 and rs2239179) modified the association of 25(OH)D with their outcome, which was a composite of incident hip fracture, myocardial infarction, cancer (excluding non-melanoma skin cancer), and all-cause mortality. However, the use of a composite outcome which combines diseases with diverse underlying pathophysiology limits clear clinical meaningfulness of these findings.

Two studies have since sought to replicate these findings. In the first study, 2 VDR gene SNPs (rs7968585 and rs2239179) did not modify the association between 25(OH)D and cardio-metabolic risk factors. Importantly, though, they examined intermediate traits (i.e. lipid and inflammatory markers), not overt CVD endpoints (16). Second, among Tromsø Study participants, VDR SNP rs7968585 did not modify associations between 25(OH)D and type 2 diabetes, myocardial infarction, cancer or overall mortality (17). Notably, all studies were restricted to Caucasian participants. VDBP SNPs (rs7047 and rs4588) have been examined in relation to serum 25(OH)D and incident stroke, heart failure and coronary heart disease in ARIC participants (9, 10, 18).

To date, it is unclear whether these vitamin D metabolic pathway SNPs modify the association between 25(OH)D and risk of cardiovascular diseases, particularly in nonwhites.

5. Main Hypothesis/Study Questions:
- To examine whether SNPs related to the vitamin D metabolic pathway modify the association between 25(OH)D and CVD risk in white ARIC participants.
  - To replicate findings by Levin et al (15) that 2 VDR SNPs modify associations of 25(OH)D with clinical outcomes in white participants.
- To examine whether these vitamin D metabolic pathway SNPs modify the association of 25(OH)D and CVD risk in black ARIC participants.

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
Prospective cohort from visit 2 through 2013
**Inclusion/Exclusion**

*Exclusions:* Those with a CVD outcome prior to visit 2, those not attending visit 2, those who are neither black nor white, blacks at the MD and MN centers, those with an estimated glomerular filtration rate (eGFR) < 15 mL/min/1.73 m², and those missing serum vitamin D concentration, genotyping, or those with incomplete information related to outcomes.

**Variables**

*Exposures:* Visit 2 serum vitamin D (adjusted for seasonality)

*Outcomes:* incident coronary heart disease, heart failure, stroke, peripheral artery disease, atrial fibrillation, and CVD mortality (individual outcomes and as composite outcome)

*Covariates:* Age, race, center, sex, educational attainment, smoking status, alcohol consumption, physical activity (sports index), BMI, systolic blood pressure, antihypertensive medication use, diabetes, total cholesterol, HDL cholesterol, lipid lowering medication use, and eGFR (measured using serum cystatin C and creatinine; categorized into ≥90, 60-90, <60 mL/min/1.73 m²)

*Effect Modifiers:* VDBP SNPs rs7047 and rs4588; VDR SNPs rs7968585 and rs2239179; CUBN SNPs rs1801222 and rs12766939; CYP27B1 SNP rs703842

**Data Analysis**

Visit 2 will serve as baseline. All analyses will be race-stratified. Baseline participant characteristics will be described by proportions or means stratified by clinically relevant serum 25(OH)D concentrations (≥30, 20-<30, <20 ng/ml) (19).

Cox proportional hazards regression (stratified by race) will be used to examine the association between 25(OH)D and risk of CVD outcomes. We will employ an additive genetic model, and (in blacks) adjust for principal components of ancestry to account for possible confounding due to population stratification. In model 1, we will adjust for age, center and sex. In model 2, we will additionally adjust for smoking status, alcohol consumption, physical activity (sports index) and BMI. In model 3, we will further adjust for systolic blood pressure, antihypertensive medication use, diabetes, total cholesterol, HDL cholesterol, lipid lowering medication use and eGFR. We will test for two-way multiplicative interactions of each SNP on the 25(OH)D-CVD association by including a cross-product term [25(OH)D*SNP] in the models (separately).

We will examine the specific CVD outcomes separately and also as a composite [incident coronary heart disease, heart failure, stroke, peripheral artery disease, atrial fibrillation, and CVD mortality]. Person-time will accrue from date of visit 2 until the specific CVD outcome, date of death, date of last contact, through December 31, 2013 (if most recent follow-up data), or whichever comes first.

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? ___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 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)?

No prior proposals have looked at interactions between 25(OH)D and these SNPs in relation to outcomes. The most similar proposals are those that look at 25(OH)D concentrations and outcomes:

<table>
<thead>
<tr>
<th>MS #</th>
<th>Outcome</th>
<th>First authors (and Senior, if student first author)</th>
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<tbody>
<tr>
<td>2019</td>
<td>Stroke</td>
<td>Schneider (Michos)</td>
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<tr>
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<td>2224</td>
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<td>Alonso</td>
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<td>2479</td>
<td>PAD</td>
<td>Rapson (Lutsey)</td>
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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* 2009.17)

____ 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/](http://publicaccess.nih.gov/) are posted in [http://www.cscs.unc.edu/aric/index.php](http://www.cscs.unc.edu/aric/index.php), under Publications, Policies & Forms. [http://publicaccess.nih.gov/submit_process_journals.htm](http://publicaccess.nih.gov/submit_process_journals.htm) shows you which journals automatically upload articles to PubMed Central.

13. Per Data Use Agreement Addendum for the Use of Linked ARIC CMS Data, approved manuscripts using linked ARIC 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 __X__ No.
References


