1.a. Full Title:

Vitamin D Receptor Polymorphisms And Carotid Intimal Medial Thickness: the Atherosclerosis Risk in Communities (ARIC) Carotid MRI Study

b. Abbreviated Title (Length 26 characters):

VDR SNPs and cIMT

2. Writing Group:

Writing group members:

Erin D. Michos, MD, MHS¹, Adrienne Tin, MS², Anna Kottgen, MD, MPH², Eric Boerwinkle, PhD³, Michal L. Melamed, MD, MHS⁴, Wendy Post, MD, MS¹,², W. Linda Kao, PhD²

1. Division of Cardiology, Johns Hopkins School of Medicine, Baltimore, MD
2. Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
3. Human Genetics Center and Institute of Molecular Medicine, University of Texas Health Science Center, Houston, TX
4. Division of Nephrology, Albert Einstein School of Medicine, Bronx, NY

FYI: Josef Coresh (Johns Hopkins) and Aaron Folsom (U of Minnesota) were both invited to participate on this proposal, but declined.

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

First author: Erin D. Michos, MD, MHS
Address: Division of Cardiology
Johns Hopkins School of Medicine
Carneige 568, 600 N. Wolfe Street
3. Timeline:

(1) Ancillary Study approved in 2006 – ancillary study approval # AS 2006.08
(2) VDR SNPs were completed in the lab of Dr. Boerwinkle
(3) Preliminary statistical analyses completed in Dec 2008
(4) Abstract draft written, planned for submission to the American Heart Association Meeting November 2009 (abstraction submission deadline June 5, 2009)
(5) Abstract approved by co-authors, was submitted to ARIC Steering Committee for review on May 7, and was tentatively approved by the ARIC Steering Committee on May 14, 2009
(6) On May 20, 2009, I was informed that I needed a manuscript proposal number in addition to my ARIC ancillary approval number. I now hope for expedited review in order to make the June 5 abstract submission deadline.

[FYI: I am very sorry – I am new to ARIC. When I submitted my proposal in 2006, I thought I was submitting a manuscript proposal. I thought this fell under the umbrella of the ARIC Carotid MRI ancillary study. When I received the approval number AS2006.08, I thought it was for the manuscript. This entire time I did not realize that my approval number was an ancillary approval number and not a manuscript proposal approval number.]

4. Rationale:

Vitamin D deficiency as measured by serum levels of 25-hydroxyvitamin D is associated with cardiovascular disease (CVD) risk. Previous studies suggested that single nucleotide polymorphisms (SNPs) in the vitamin D receptor (VDR) gene, the functional significance of which are debated, are associated with CVD. In particular the “B” allele of rs1544410 (described by BsmI restriction fragment cleavage) was associated with myocardial infarction and higher carotid intimal medial thickness (cIMT) compared to “b” allele. However, no studies have comprehensively examined the association of other VDR
SNPs, which may be in linkage disequilibrium (LD) with rs1544410, with cIMT, a marker of subclinical vascular disease. Furthermore, previous studies testing the association of VDR polymorphisms with cIMT were performed in Caucasian populations, and have not been evaluated in African Americans – a race/ethnicity with the greatest prevalence of vitamin D deficiency. Perhaps some of the discrepancy in results of studies associating 25(OH) vitamin D levels with CVD risk may be explained by VDR gene polymorphisms.

5. Main Hypothesis/Study Questions:

Our hypothesis is that rs1544410 will be associated with cardiovascular risk, as measured by increased cIMT, as found in other studies from Caucasian populations. This association will be confirmed as the previous nomenclature in the literature based on Bsm I restriction fragment cleavage is now outdated. Whether the association of rs154410 with cIMT will be found among African American race/ethnicity will be evaluated in this study. Additionally we seek to evaluate whether any additional vitamin D receptor SNPs, other than the rs1544410 (i.e. “B” allele), have any strong associations with cIMT. Since rs1544410 is located within an intron and is thus thought not to be functionally significant, it may be in LD to a more functional mutation.

[Note: This ancillary study will serve as preliminary data for preparing future grant proposals. Since VDR SNP will be measured in all of ARIC as part of the CARE consortium, future proposals will be generated proposing to look at the association of VDR SNPs with CVD events in the full ARIC cohort. In addition, a grant proposal is being prepared proposing to measure serum 25-hydroxyvitamin D levels in a subset of ARIC participants, to determine the interaction between VDR genetics and serum vitamin D levels on CVD risk. These ancillary and manuscript proposals will be submitted separately.]

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).

ARIC is a community-based prospective cohort of 15,792 white and black adults aged 45-64 years in 1987-89. The ARIC Carotid MRI ancillary study includes a subset of 1250 cases with the thickest maximal cIMT (representing the 73rd, 69th, 73rd, and 69th percentiles at Forsyth County, Jackson, Minneapolis suburbs, and Washington County, respectively) and 816 randomly selected controls that underwent additional study in 2005-06 including carotid MRI and genotyping. Further details regarding the ARIC Carotid MRI ancillary study can be found on the approved study proposal.
Our approved ancillary study (AS 2006.08) proposed to evaluate the association of vitamin D receptor (VDR) polymorphisms with cIMT (case/control status) among participants in the ARIC Carotid MRI ancillary study.

The algorithm used for the SNPs selection was Haploview's implementation of the Broad Institute's Tagger software. The R squared cut off for Tagger was set to 0.8 and the LOD threshold to 2. In addition, Tagger was used in aggressive multi-marker mode. SNPs with a minor allele frequency (MAF) of less that 0.05 were excluded from consideration before the tag SNPs were calculated. A limited number of additional candidate SNPs were included if provided by an ARIC investigator. The final SNP set for each gene was determined by taking the union of the four SNP sets (nonsynonymous, tagSNPs from each population and PI requested SNPs) for each gene. The overall SNP set is time-dependent and is likely to change as the data at the various SNP databases in refined or expanded. At the time this SNP panel was created we used Haploview v. 3.32pr, Hap Map Data Rel 22/phase Apr 07 and NCBI 36/dbSNP 126.

In summary, final VDR SNP panel include those independently selected from the HapMap Caucasians (n=27) and Yorubans (n=7), the non-synonymous SNPs (n=3), and the specifically requested SNPs from various PI's (n=1, the rs1544410 per my request), for a total of 38 VDR SNPs. After assay design and laboratory quality control was complete, 31 SNPs from VDR passed. The assay used for genotyping was the Illumina iSelect. Genotyping was performed in Dr. Eric Boerwinkle’s lab at University of Texas Health Sciences Center in Houston.

Allelic and genotypic frequencies will be used to test for departures from Hardy-Weinberg equilibrium (HWE), stratified by race/ethnicity. SNPs indicating significant departure from HWE (p<0.01) will be flagged and genotype calling will be double checked. If genotyping error is accountable for the departure, then the SNP will be thrown out. SNPs that persist out of H-W proportions after testing for genotyping error will be kept in the analyses as they may provide valuable insight into population ancestry, or signal a genome region for which the study sample is biased or is under selection pressure. Population estimates of the minor allele frequencies (MAFs) will be estimated in African Americans and Caucasians separately. Patterns of linkage disequilibrium will be determined using standard estimates of D’ and r² and compared with that from the International HapMap Project using the program Haploview. Both genotype- and haplotype-based analyses will be performed.

The association of each VDR SNP with cIMT case/control status will be evaluated using logistic regression analyses stratified by race. Unadjusted models will be performed first, followed by limited adjusted models adjusting for center, age, sex. Finally multivariable models will be performed including additional adjustment for hypertension, smoking, diabetes, BMI, physical activity, GFR, total and HDL cholesterol. Covariates for inclusion in multivariable models were chosen a priori because of association with serum vitamin D levels and CVD risk. To account for multiple testing, an association will be considered statistically significant based on a Bonferroni-corrected alpha [0.05/31 SNPs or p<0.0016.]
Power calculations using PS power and sample size program revealed that this study will have 80% power to detect an odds ratio of 1.29 or less assuming that the SNP being examine dis in complete linkage disequilibrium with the causal variant that the minor allele frequency is 0.44 at an $\alpha$ of 0.05.

7.a. Will the data be used for non-CVD analysis in this manuscript?  
____ Yes    _X_ 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?  
_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

**VDR SNPS were not run on those who reported “no use/storage DNA”**

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](http://www.cscc.unc.edu/ARIC/search.php)

____ Yes    _______ No    ___X__ Possible overlap

Our ancillary study to look at VDR SNPs and cIMT was approved in 2006. Since then, there was an ARIC manuscript proposal #1405 (7/30/08) proposing to look at genewide associations with log-transformed cIMT as part of the CHARGE GWAS collaboration. That proposal does not specifically focus on particular candidate genes such as the VDR SNP.

Thus I believe there is not significant overlap with my proposal. Vitamin D receptor genotyping was only done among participants in the Carotid MRI ancillary study, not the whole cohort. We are looking at the association of one particular candidate gene as it relates to case (thickest cIMT)/ control status.
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)?

As above, the most closely related proposal is #1405 “Subclinical Measures GWA Collaboration: Carotid Intima-Media Thickness”

Eric Boerwinkle is a coauthor on our proposal and can ensure that there is no overlap. Anna Kottgen is also a co-author on our proposal and #1405.

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* __AS 2006.08__ - "The Association of Vitamin D Receptor B/b Polymorphism with Subclinical Cardiovascular Disease Risk: The ARIC study."____)

   ____  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/

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.