ARIC Manuscript Proposal #2853

1.a. Full Title: Serum vitamin D, vitamin D-related genetic variation, and prostate cancer in the Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): Vitamin D and Prostate Cancer

2. Writing Group:
   Writing group members: Alison Mondul, Elizabeth Platz, Corinne Joshu, Pamela Lutsey, Erin Michos, Anna Prizment
   Invited: other ARIC investigators are welcome

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

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3. Timeline: The proposed project is an analysis of existing data. We anticipate the analysis of the existing data and drafting of the manuscript will take 12 months from the time of manuscript approval.

4. Rationale:
It has been hypothesized that vitamin D may prevent many types of malignancies including prostate cancer. The primary circulating form of vitamin D is total 25-hydroxyvitamin D (25(OH)D), which is comprised of 25(OH)D$_2$ and 25(OH)D$_3$, and is considered the best indicator of an individual’s vitamin D status. 25(OH)D is converted to the hormonally active form, 1-25-dihydroxyvitamin D (1,25(OH)$_2$D), by 1-α-hydroxylase. Laboratory studies have demonstrated that 1,25(OH)$_2$D has many anti-carcinogenic actions, including being anti-inflammatory, anti-angiogenic, and pro-apoptotic. Evidence from epidemiologic studies with measured circulating 25(OH)D concentration is inconsistent with respect to the role of vitamin D in prostate cancer, however. A recent meta-analysis concluded that men with higher serum 25(OH)D had a higher risk of developing cancer of the prostate than men with lower serum 25(OH)D, although this analysis did not differentiate between more and less aggressive disease. Two recent studies suggest that the association differs by aggressiveness, with one reporting a possible U-shaped relation with plasma 25(OH)D, and the other indicating higher 25(OH)D might be associated with an increased risk of low-grade, but a decreased risk of high-grade, disease.

Prostate cancer is the most commonly diagnosed cancer among men in the US, but has one of the most favorable survival rates, with 94% of patients surviving for at least 15 years. Thus, prostate cancer mortality is perhaps a more clinically relevant outcome for risk association analyses. Yet few studies have examined serum 25(OH)D in relation to this endpoint, and those that have produced conflicting results. Three studies examined pre-diagnostic 25(OH)D concentrations comparing incident cases of lethal prostate cancer to cancer-free controls; one found an inverse association while two others showed no association. In a recent study published by the lead author of this proposal, Dr. Mondul, higher pre-diagnostic serum 25(OH)D concentration was associated with lower prostate cancer-specific mortality among men diagnosed with prostate cancer in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study. In two studies, serum 25(OH)D concentration at the time of prostate cancer diagnosis was unrelated to subsequent mortality, but was inversely associated in one study. Thus, there remains a lack of clarity with respect to the role of vitamin D in prostate cancer, particularly with respect to disease aggressiveness and death from prostate cancer.

Another important, but understudied, area is the role of vitamin D in prostate cancer among African American men. Due to variation in skin pigmentation by race, it is known that African Americans tend to have lower circulating 25(OH)D levels than Whites. A recent study in the National Health and Nutrition Examination Survey (NHANES) reported that the mean 25(OH)D concentration in White US adults was 26 ng/mL, whereas it was 15 ng/mL among African American adults, and some researchers have suggested that lower vitamin D status among African Americans may partially explain Black-White health disparities in the United States, including the 2-3-fold higher risk of prostate cancer in Black vs. White men. However, this remains an understudied area with most of the vitamin D-prostate cancer studies to date having been conducted in Caucasian men.
Two recent genome-wide association studies (GWAS) identified SNPs (including two not previously well-known) in or near four genes related to circulating 25(OH)D \textsuperscript{18, 19}. The four genes identified were: \textit{GC}, which encodes vitamin D binding protein (DBP), the major carrier of vitamin D compounds in circulation; \textit{CYP24A1}, which encodes the cytochrome p450 (CYP) 24-hydroxylase that initiates intracellular metabolism of 25(OH)D and 1,25(OH)\textsubscript{2}D to less bioactive species; \textit{CYP2R1}, which encodes a key 25-hydroxylase responsible for conversion of vitamin D to 25(OH)D in the liver; and, \textit{DHCR7}, which encodes the enzyme that catalyzes the conversion of 7-dehydrocholesterol, a vitamin D\textsubscript{3} precursor, to cholesterol \textsuperscript{18, 19}. Dr. Mondul has previously published a study examining variation in these four SNPs in relation to overall and aggressive prostate cancer among Caucasian participants in the Breast and Prostate Cancer Cohort Consortium (BPC3) \textsuperscript{20}. Corroboration of these results in a different study population, particularly one that includes African Americans, is warranted.

5. \textbf{Main Hypothesis/Study Questions:}

\textbf{Question 1:} Is serum total 25(OH)D associated with risk of overall, lethal phenotype, and fatal prostate cancer? We hypothesize that higher serum total 25(OH)D will be associated with an increased risk of overall prostate cancer, but a lower risk of lethal phenotype disease and fatal prostate cancer.

\textbf{Question 2:} What are the associations between 25(OH)D\textsubscript{2}, 25(OH)D\textsubscript{3}, and 3-epi-25(OH)D\textsubscript{3} and overall, lethal phenotype, and fatal prostate cancer? Given that the majority of total 25(OH)D is 25(OH)D\textsubscript{3}, we hypothesize that any vitamin D association with prostate cancer will be driven by 25(OH)D\textsubscript{3}. Analyses of 25(OH)D\textsubscript{2} and the 3-epi-25(OH)D\textsubscript{3} epimer are exploratory and will likely have limited power.

\textbf{Question 3:} What are the associations between the circulating vitamin D-associated SNPs identified by GWAS and risk of overall, lethal phenotype, and fatal prostate cancer? We hypothesize that, as proxies for total circulating 25(OH)D concentration, these SNPs will have a similar association as that for serum 25(OH)D in Question 1.

\textbf{Question 4:} Do any of the studied associations differ between White and Black men? We hypothesize that vitamin D will be associated with risk of prostate cancer in both racial groups, although the burden of lethal phenotype and fatal prostate cancer will be higher in Black men because of higher prevalence of low vitamin D.
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

**Analysis:** Cox proportional hazards models with age as the time metric.

**Exclusions:** Women, men who had cancer prior to measurement of circulating vitamin D at Visit 2, men who were missing cancer information prior to measurement of circulating vitamin D at Visit 2, men with missing exposure information.

**Exposures:** Serum 25(OH)D$_2$, 25(OH)D$_3$, 3-epi-25(OH)D$_3$ measured at Visit 2 (1990-1992). Serum 25(OH)D$_2$ and 25(OH)D$_3$ will be examined separately, as well as combined to reflect total serum 25(OH)D. 3-epi-25(OH)D$_3$, 25(OH)D$_2$, 25(OH)D$_3$, and total 25(OH)D will be modeled as both quantiles (either quintiles or quartiles) and as continuous variables. We realize that concentrations of 3-epi-25(OH)D$_3$ and 25(OH)D$_2$ are likely to be low, so our main exposure is total 25(OH)D. Further, total 25(OH)D will be categorized according to a priori cutpoints based on the Institute of Medicine definitions for “deficiency” (<30 nmol/L), “possible inadequacy” (30 - <50 nmol/L), “adequacy” (50 - <75 nmol/L), and “beyond adequacy” (≥ 75 nmol/L), and we will explore restricted cubic spline models, as well, because the association may not be linear. Because 25(OH)D concentrations are known to vary by season of blood collection, and participants were enrolled in the study throughout the year, we will use two approaches to address the influence of season. First, we will determine quantile cutpoints separately for participants whose blood was collected in the winter (November – May) and in the summer (June – October) based on the distribution in of blood values in each season. Second, we will perform season standardization of the various measured 25(OH)D values using a periodic sine/cosine function.

Genotype for the following vitamin D-related SNPs rs2282679, rs6012897, rs12785878, rs10741657. These SNPs will be coded based on the number of alleles (0, 1, 2) associated with lower circulating 25(OH)D levels (i.e., low vitamin D alleles) in the two published GWAS studies of serum 25(OH)D concentrations$^{18,19}$, rather than on the number of minor alleles. Additionally, all four SNPs will be combined to create a vitamin D score ranging from 0-8 low vitamin D alleles. Because few men will likely have 0, 7, or 8 low vitamin D alleles, those with 6, 7, or 8 alleles will likely be merged into one category, as will those with 0 or 1 alleles. Individual SNPs and the risk score will be analyzed in two ways. First, by entering separate indicator variables for the number of low vitamin D alleles into the regression model using 0 alleles as the referent group for the individual SNP analyses and using 0-1 alleles as the referent group for the risk score analysis. Second, by including in the model the ordinal variable for the number of low vitamin D alleles ranging from 0-2 each for the individual SNP analyses and from 0-8 for the risk score analysis to estimate the per-allele difference in risk of prostate cancer. This approach was successfully used in two published analyses by the lead author in the Breast and Prostate Cohort Consortium (BPC3)$^{20,22}$. 
**Outcome:** Incident prostate cancer through 2012. This will be defined several ways: overall prostate cancer, lethal phenotype (metastatic or fatal), and fatal disease (defined as men who died from their prostate cancer).

**Covariates:** Age, race, field center, waist-hip ratio, body mass index, height, diabetes (self-reported and/or based on glucose measurements), cardiovascular disease co-morbidities, smoking, alcohol consumption, physical activity, education level, dietary factors (particularly dietary vitamin D and calcium intake), aspirin and other NSAIDS, medications to treat diabetes (particularly metformin), family history of cancer, other health screenings including an annual physical examination (as a surrogate for PSA screening, which is not available).

**Stratification Variables:** We will stratify by race, season of blood collection, and BMI.

**Limitations:** Although the most clinically relevant outcome for prostate cancer is lethal or fatal disease, there are currently only ~90 fatal cases in the ARIC cohort, which will limit our ability to conduct stratified analyses.

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 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](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)?

Many manuscript proposals use the vitamin D analytes and related SNPs. With respect to cancer the following are relevant:
2064 vitamin D and colorectal cancer (Prizment, plus Joshu, Platz, Nelson, Robin, Folsom from ARIC Cancer)

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* 2011.07, 1995.04, 2009.17, 2010.01)

___ 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.cscu.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. AMM

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.cscu.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. AMM

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  _X__ No.
References


