

ARIC Manuscript Proposal #2198

PC Reviewed: 8/13/13
SC Reviewed: _____

Status: A
Status: _____

Priority: 2
Priority: _____

1.a. Full Title: Mineral Metabolism Biomarkers Associated with Risk of End-Stage Renal Disease in a Nested Case-Control Study: CKD Biomarkers Consortium

b. Abbreviated Title (Length: 18 characters): Vitamin D and ESRD

2. Writing Group:

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

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3. Timeline: Data analysis and manuscript preparation will begin immediately. We anticipate submitting a draft for ARIC review within 3-6 months of proposal approval and receipt of the final dataset.

4. Rationale:

Disordered mineral metabolism is characteristic of impaired kidney function, particularly in the early stages of chronic kidney disease (Craver *et al.*, 2007). Declining 1α -hydroxylase activity in the kidney results in decreased activation of vitamin D, leading to hypocalcemia and hyperparathyroidism (Craver *et al.*, 2007). Fibroblast growth factor-23 (FGF-23) is a bone-derived hormone with several endocrine functions in the renal proximal tubule, including the induction of urinary phosphorus excretion, inhibition of vitamin D activation, and suppression of parathyroid hormone synthesis (Shimada *et al.*, 2004). Low levels of vitamin D is a therapeutic target for kidney disease patients, however, the clinical recommendation to replace nutritional vitamin D is opinion-based and needs additional empirical evidence (National Kidney Foundation, 2003; Kandula *et al.*, 2011). FGF-23 and vitamin D, as early markers of kidney injury, may be useful in risk stratification for kidney disease outcomes.

Previous studies have been inconsistent in their methods for quantifying vitamin D levels. In the published literature, total vitamin D levels are usually reported. The majority of circulating vitamin D (85-90%) is tightly bound to vitamin D binding protein, a smaller amount (10-15%) is loosely bound to albumin, and less than 1% is circulating in a free, unbound form (Bikle *et al.*, 1985). The free form is thought to be the most biologically active, followed by the vitamin D loosely bound to albumin (Mendel, 1989). Bioavailable vitamin D refers to the free form and vitamin D bound to albumin, and can be estimated as the difference between total vitamin D and vitamin D binding protein (Bhan *et al.*, 2012). Although ergocalciferol (D_2 ; derived from consumption of plants and dietary supplements) and cholecalciferol (D_3 ; derived from consumption of animal productions, dietary supplements, and produced in the skin) were previously thought to be biologically equivalent, it is now known that D_3 is at least 2-times more potent in changing levels of active vitamin D (Heaney *et al.*, 2011; Trang *et al.*, 1998). Estimation of disease risk using bioavailable vitamin D, and vitamin D_3 specifically, is particularly relevant and novel, and could be compared to risk estimates using traditional measurements of vitamin D (*i.e.* total vitamin D).

An increased risk of end-stage renal disease (ESRD) with lower levels of 25-hydroxy-vitamin D has been reported in a small chronic kidney disease clinic study (N=168) and among participants of the third National Health and Nutrition Examination Survey (Melamed *et al.*, 2009; Ravani *et al.*, 2009). In the Chronic Renal Insufficiency Cohort study of 3,879 participants, FGF-23 was independently associated with risk of ESRD among those with GFR 30-44 mL/min/1.73m² and ≥ 45 mL/min/1.73m², but not among those with GFR < 30 mL/min/1.73m² (Isakova *et al.*, 2011). The relationship between vitamin D binding protein, vitamin D levels, and associated biomarkers (FGF-23) with incident ESRD has not been extensively evaluated in epidemiologic studies. We propose to assess the association between levels of vitamin D, vitamin D binding protein, and FGF-23 with incident ESRD in a case-control study nested within the Atherosclerosis Risk in Communities study, a community-based prospective cohort study, after adjusting for measure of kidney function (GFR) and other known ESRD risk factors.

5. Main Hypothesis/Study Questions:

- (1) We hypothesize that vitamin D binding protein and fibroblast growth factor-23 will be positively and independently associated with risk of ESRD.
- (2) We hypothesize that total vitamin D will be negatively and independently associated with risk of ESRD.

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: Nested case-control study within the Atherosclerosis Risk in Communities study, a community-based prospective cohort study with study visit 4 as baseline

Participants (Inclusion/Exclusion, Case and Control Selection, Matching Criteria):

Study participants were excluded for the following reasons: 1) ESRD diagnosed prior to ARIC study visit 4; 2) eGFR was missing at ARIC study visit 4; 3) ACR was missing at ARIC study visit 4; 4) diabetes status was missing at ARIC study visit 4; 5) African-American race from study centers in Minneapolis, Minnesota and Washington County, Maryland; and 6) Caucasian race from study center in Jackson, Mississippi.

All cases and controls were selected from the ARIC cohort. ESRD cases were identified through hospitalization surveillance from baseline at ARIC study visit 4 (1996-1998) through December 31, 2008. Controls were frequency matched to cases based on CKD-EPI estimated glomerular filtration rate (eGFR) category (<45, 45-59, 60-74, 75-89, 90-105, ≥ 105 mL/min/1.73 m²), urinary albumin-to-creatinine ratio (ACR) category (<30, 30-299, ≥ 300 mg/g), diabetes status (fasting glucose >126 mg/dL, self-reported diabetes medication use in the past two weeks, or self-reported diagnosed diabetes), sex, and race (African-American, Caucasian).

We identified 184 incident ESRD cases and 251 matched controls (total N=435) with available serum samples.

Exposure: Levels of 25-hydroxy vitamin D₂, 25-hydroxy vitamin D₃, 25-hydroxy vitamin D₃ epimer, vitamin D binding protein, and FGF-23 were measured in serum specimens collected during ARIC study visit 4 (1996-1998), stored at -70°C at Baylor University until laboratory analysis, and shipped to the Advanced Research and Diagnostics Laboratory at University of Minnesota for laboratory analysis (March-April 2013). Intact FGF-23 and vitamin D binding protein were measured using commercially available ELISAs (intact FGF-23: Kainos Laboratories, Inc., Kyoto, Japan; vitamin D binding protein: human Quantikine®, R & D Systems, Inc., Minneapolis, MN). The remaining biomarkers – 25-hydroxy-vitamin D₂, 25-hydroxy-vitamin D₃, and vitamin D₃ epimer – were measured by liquid chromatography/tandem mass spectrometry. If possible, 1,25-dihydroxyvitamin D and albumin will also be measured in serum samples from cases and controls.

Outcome: The outcome of interest is incident ESRD, defined by hospitalization surveillance between baseline at ARIC study visit 4 (1996-1998) through December 31, 2008. ESRD case status was defined by: 1) death certificates with renal failure ICD codes (584-584.9, 586, N17.0) as the underlying cause of death and history of chronic kidney disease, indicated by creatinine rise, eGFR MDRD, or surveillance ICD code; or 2) hospitalization ICD codes for kidney transplant, dialysis, or procedural code indicating dialysis, except for hospitalizations with ICD code for traumatic anuria (958.5) on the same hospitalization date, or acute renal failure hospitalization ICD codes (586, 788.9) without prior chronic kidney disease events. This outcome definition has been used previously in an ARIC study (Bash *et al.*, 2010). In addition, a validation study of kidney disease outcomes is currently being conducted in the ARIC study.

Other Variables of Interest: The following variables measured during ARIC study visit 4 will be considered for adjustment in multivariable regression models and for stratification:

- Matching factors: eGFR, ACR, diabetes status, sex, race
- Potential covariates/mediators: age, systolic blood pressure, diastolic blood pressure, hypertension status, body mass index, smoking status, history of coronary heart disease, use of supplemental vitamin D (prescribed and over-the counter), season of specimen collection, and study center.

For the purpose of creating strata to match cases to controls and adjustment in multivariable models, eGFR will be calculated using the Chronic Kidney Disease Epidemiology Collaboration equation (Levey *et al.*, 2009). For defining case status, MDRD eGFR as reported in hospital records will be utilized (Levey *et al.*, 1999).

Statistical Analysis:

The distribution of the biomarkers of interest (vitamin D, vitamin D binding protein, and FGF-23) will be assessed by visual inspection of histograms and statistical tests of normality. If the distribution is not normal, transformation will be considered [$\log_{10}(x+1)$]. The biomarkers of interest will be expressed continuously, in quantiles, and categorized based on clinical guidelines, when available (dichotomized vitamin D: <30 and >30 ng/mL, 3 categories of vitamin D: <20, 20-30, and >30 ng/mL, using the highest category as the reference group for estimating risk ratios). If we are able to measure albumin, free and bioavailable vitamin D levels will be calculated (Bhan *et al.*, 2012; Powe *et al.* 2011).

Cases and controls will be compared based on the matching factors (eGFR, ACR, diabetes status, sex, race). If the cases and controls differ substantially on the matching factors, additional adjustment in multivariate models will be considered.

For descriptive purposes, we will examine characteristics of study participants by case status. We will use means and proportions to describe the participants and test for differences using χ^2 and t tests.

Means (standard deviations) and medians (inter-quartile ranges) will be calculated for all biomarkers of interest by case status. Potential covariates for adjustment include: eGFR (continuous and spline with knot at 60 mL/min/1.73 m²), ACR, diabetes status, sex, race, age, systolic blood pressure, diastolic blood pressure, hypertension status, body

mass index, smoking status, history of coronary heart disease, use of supplemental vitamin D (prescribed and over-the-counter), season of specimen collection, and study center. The distribution of the biomarker values will be plotted by case status.

Since serum vitamin D levels vary greatly by sun exposure, which is seasonal, we will account for seasonal variation by computing the residuals from a linear regression model with vitamin D as the dependent variable and month of blood draw as the independent variable. By definition, these residuals will be uncorrelated with month of blood draw. The grand mean will be added to the vitamin D residuals obtained from this model. This new variable “vitamin D adjusted for month of blood draw” will be used as the main exposure variable for all analyses.

The incident odds ratios (95% confidence intervals) for ESRD will be estimated using crude and adjusted conditional logistic regression models to account for matching and unconditional logistic regression models. Unconditional logistic regression models will additionally be adjusted for matching factors. Odds ratios and 95% confidence intervals will be estimated by quartile, by clinically-relevant category (for vitamin D only), and for an increase in inter-quartile range and standard deviation in biomarkers. Associations will be estimated for vitamin D₂ and vitamin D₃ separately as well as for total vitamin D (sum of D₂ and D₃). The influence of season and location (study center) of specimen location will be assessed by bivariate comparison with vitamin D levels and stratified risk estimated by these factors.

Limitations: There may be bias introduced by overmatching, which, if present, would be expected to underestimate the risk ratios. The serum specimens were stored for approximately 15 years before laboratory measurement and thus may be subjected to degradation, albeit non-differential by case status. Previous research has documented the stability of serum levels of vitamin D with a long duration of freezer storage (Agborsangaya *et al.*, 2010). Loss to follow-up is a potential source of selection bias in the study. The discussion section of the manuscript will mention these limitations in the context of the study strengths.

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? ___
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?
___ 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"?

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.csc.unc.edu/ARIC/search.php>

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)?

#2152 – Vitamin D and venous thromboembolism

#2143 - Association of fibroblast growth factor-23 levels with risk and progression of chronic kidney disease: the Atherosclerosis Risk in Communities (ARIC) study

#2108 - FGF-23 and incident coronary heart disease, heart failure, and total mortality

#2088 - FGF-23 and atrial fibrillation

#2066 – Associations between vitamin D status and diabetic retinopathy in a biracial cohort

#2064 – Vitamin D, parathyroid hormone (PTH) and fibroblast growth factor (FGF) 23 in relation to colorectal cancer risk and mortality in the Atherosclerosis Risk in Communities Study

#2021 – Vitamin D and neurocognitive decline: the ARIC brain ancillary study

#2020 – Vitamin D and subclinical cerebrovascular disease: an ARIC brain MRI ancillary study

#2019 – 25-OH vitamin D levels and incident stroke: 20 year follow-up in a biethnic cohort

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? Yes No

11.b. If yes, is the proposal

A. primarily the result of an ancillary study

List numbers*:

2009.31: Longitudinal Study of Predictors and Consequences of Chronic Kidney Disease: Proteomic Approach to CKD Biomarker Discovery and Validation

2006.16: Longitudinal Study of Predictors and Consequences of Chronic Kidney Disease

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.csc.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.csc.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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