ARIC Manuscript Proposal #2502

PC Reviewed: 3/10/15	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Association of mid-life Vitamin D levels, Vitamin D Binding Protein Genetic Polymorphisms and Race with Later Life Performance on Neuropsychological Testing: the Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): Vitamin D and Neuropsychological performance

2. Writing Group: Writing group members:

Erin D. Michos	Johns Hopkins University -approved
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I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. __EM__ [**please confirm with your initials electronically or in writing]**

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:

Vitamin D lab assays from ARIC visit 2 have been completed. Plan to work on this proposal immediately pending approval.

4. Rationale:

Low serum 25-hydroxyvitamin D [25(OH)D] levels are associated with coronary heart disease,[1] stroke,[2] and its risk factors such as hypertension and diabetes.[3] Emerging data suggest that vitamin D may be important for cognitive functioning and may be protective against neurovascular injury.[4] Vitamin D receptors are located in the human cortex and hippocampus, areas of the brain important for cognitive functioning, and vitamin D receptor down regulation in these key areas has been associated with Alzheimer's disease.[4] Therefore adequate levels of vitamin D in midlife may help prevent cognitive decline and dementia, particularly Alzheimer's and vascular dementia.

We previously analyzed the association of 25(OH)D levels measured during the ARIC visit 3 (1993-1995) with cognition among 852 whites and 800 blacks participants of the ARIC Brain MRI ancillary study from the Jackson and Forsyth County sites.[5] We found that lower 25(OH)D was not associated with 3 cognitive measures (DWRT, DSST, WFT) cross-sectionally at visit 3, nor with greater DWRT, DSST, or WFT decline for both short term follow-up (visit 3 to visit 4) and ~10-year follow-up (visit 3 to the 2004-2006 ARIC Brain MRI visit) ($p>0.05$ for all associations). Though not statistically significant, lower levels of 25(OH)D were suggestive of an association with increased dementia risk (HR lowest versus highest race-specific tertile: whites 1.32 [95% CI: 0.69, 2.55]; blacks 1.53 [95% CI: 0.84, 2.79]). We concluded that, in contrast to prior studies performed in older white populations, our study did not find significant associations between lower levels of 25(OH)D measured in late-middle age black or white participants with lower cognitive test scores at baseline and change in scores over time. However in this paper, we did not use ARIC-NCS data, nor did we examine a more comprehensive approach to neuropsychological testing. We also did not take into account possible biases due to selective attrition.

A primary objective of the Atherosclerosis Risk in Communities Brain MRI study (ARIC Brain) held in 2004-2006 and the ARIC Neurocognitive study (ARIC NCS) held in 2011-2013 is to evaluate the contribution of vascular risk factors, measured during midlife, to long-term cognitive decline and neuropsychological functioning. The ARIC Brain Visit was a subset of the ARIC cohort at the Jackson and Forsyth County locations (~1100 individuals), while the ARIC NCS study included ~6500 individuals who were survivors of the original ARIC cohort. At these visits (ARIC Brain and ARIC NCS), a more comprehensive battery of neuropsychological tests were performed which represent 4 cognitive domains: **Memory** (*Delayed Word Recall Test, Logical Memory Test Part I and II, Incidental Learning),* **Language and Verbal Fluency** (*Animal Naming, Boston Naming Test, Word Fluency Test)*, and **Processing Speed and Executive Function** (*Trail Making Test A and B, Digital Symbol Substitution Test, Digit Span Backwards)* and **General performance** (*all of the above*) [see **supplemental table 1** for factor loading]. Rather than evaluating the association of 25(OH)D with each of the individual 10 tests, it makes more biological sense to consider the association of 25(OH)D with these 4 global cognitive domains as has been done previously in ARIC-NCS by Rawlings et al.[6]

However, one problem in ARIC is that the cognitive tests performed in ARIC differed across the study visits. Some of the tests performed in ARIC Brain differed slightly from ARIC NCS, and only DWRT, DSST, and WFT were performed at earlier ARIC visits. *For example, ARIC visit 2*

did not perform the more advanced neuropsychological testing that was done at the ARIC Brain and ARIC-NCS visits. Gross and colleagues developed a factor analysis in ARIC to use all available cognitive data to derive scores of cognitive performance to study associations with cognitive decline in these 4 areas of general cognitive performance, memory, executive functioning, and language.[7]

While vitamin D was measured at an earlier time point (ARIC visit 2) and is not contemporaneous with either the ARIC Brain or ARIC NCS visits, this is a strength of the proposed analysis rather than a limitation because of the minimization of the problem of reverse causation, e.g., where people who are more cognitively impaired may spend less time outdoors with reduced sunlight exposure (i.e. vitamin D). If vitamin D and cognitive performance is causal, then adequate levels earlier in life may be key to prevention of cognitive decline later in life.

Another area of interest is whether race modifies the association between vitamin D and neuropsychological performance. While blacks are known to have lower levels of total 25(OH)D on average compared to whites, recent work has shown that blacks and whites have similar concentrations of estimated bioavailable 25(OH)D, secondary to blacks having lower levels of both total 25(OH)D and vitamin D binding (DBP) protein compared to whites.[8] There are two common single nucleotide polymorphisms (SNPs), rs7041 and rs4588, that are associated with the DBP gene. Blacks have been shown to be more likely than whites to have a T allele at rs7041 and to have a C allele at rs4588. The rs7041 G versus T allele and the rs4588 A versus C allele have been shown to genetically predispose individuals to higher DBP levels and thus those with lower levels of bioavailable vitamin D. The DBP SNPs are also highly correlated with total 25(OH)D levels.[9] Bioavailable vitamin D may better correlate with cognitive performance than total 25(OH)D. While we do not currently have bioavailable vitamin D in ARIC, we can test for interactions by DBP SNP as a surrogate for bioavailable vitamin D status.

This new analysis is novel by using multiple tests contributing to each of the 4 cognitive domains, and we can account for attrition which may have biased our previous results toward the null.

5. Main Hypothesis/Study Questions:

1. To determine whether 25(OH)D levels measured in mid-life (years 1990-1992, population mean age 57) are independently associated with later cognitive performance on key neuropsychological variables assessed at ARIC Brain (2004-2006, population mean age 70) and the ARIC NCS (2011-2013, population mean age 77). [We will first confirm that vitamin D is not associated with cognitive function cross-sectionally at ARIC visit 2 as measured by DWRT, DSST, and WFT. The more advanced neuropsychological tests were not done at visit 2].

   <u>Hypothesis:</u> After accounting for attrition, low vitamin D status in mid-life predicts poorer performance on neuropsychological testing performed in older age.

2. For the subset that attended both the ARIC Brain visit and ARIC NCS (n~600), 25(OH)D levels measured in mid-life would be independently associated with less decline in performance between the ARIC Brain visit (2004-2006) and ARIC-NCS (2011-2013)

   Hypothesis: After accounting for attrition, low vitamin D status in mid-life is associated with more decline in neuropsychological function between ARIC Brain and ARIC NCS.

3. To determine whether the association of 25(OH)D with neuropsychological performance differs by race in both the non-concurrent cross-sectional analyses and the change analyses.

   Hypothesis: Associations of low vitamin D with cognitive performance and cognitive change will be similar by race at the ARIC Brain and ARIC NCS visits – no racial interaction.

4. To determine whether the association of 25(OH)D with neuropsychological performance differs by key DBP SNP status.

   Hypothesis: Performance on neuropsychological tests will be modified by rs7041 and rs4588 SNPs (worse performance with rs7041 G versus T allele and rs4588 A versus C allele, i.e. those genetically predisposed to higher DBP levels and thus those with lower levels of bioavailable 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: Non-concurrent cross-sectional analysis using vitamin D measured at ARIC visit 2 (mid-life) with later life neuropsychological performance at ARIC Brain visit (2004-2006), which is a subset of ARIC participants participating in this ancillary study, and ARIC-NCS (2011-2013). For those participating in both ARIC Brain and ARIC-NCS, change analyses will also be evaluated between these 2 studies.

Participants: All white and black (excluding blacks from Minnesota/Maryland) ARIC participants with 25(OH)D measured at ARIC visit 2 and neuropsychological testing at ARIC Brain and/or ARIC-NCS. We will exclude blacks from Minnesota/Maryland, those with missing genetic data (vitamin D binding protein single nucleotide polymorphisms: rs7041 and rs4588), and those missing covariates on key demographics.

We additionally will exclude data from individual study visits (not participants) when the individual reports taking CNS altering medications that may affect cognitive test performance.

Vitamin D variables: This proposal makes use of the newly measured 25(OH)D2 and 25(OH)D3 levels measured from serum samples and stored at -70^{o}C until analyzed using liquid chromatography-tandem high-sensitivity mass spectrometry (Waters Alliance e2795, Milford,

Massachusetts) in 2012-2013. The inter-assay coefficient of variations (CVs) for 25(OH)D2 is 10.9% and for 25(OH)D3 is 6.2%. 25(OH)D2 and 25(OH)D3 were added together for total 25(OH)D concentration.

Seasonally adjusted 25(OH)D: 25(OH)D concentrations vary by season. Therefore will we adjust 25(OH)D for seasonal change by computing the residuals from a linear regression model with 25(OH)D as the dependent variable and month of visit as the independent variable. The residuals will be added back to the grand mean to determine an estimated annual 25(OH)D value. We will perform this adjustment separately for whites and for blacks, as 25(OH)D concentrations also vary by race. This vitamin D adjusted for month of visit will be used in all analyses.

In analyses, we will model vitamin D both continuously and also by clinical cutpoints, with considering levels less than 20 ng/ml as deficient and >=20 ng/ml as replete.

Vitamin D binding protein SNPs (rs7041 and rs4588): We will categorize rs7041 as TT (reference) versus TG/GG and rs4588 as CC (reference) versus AC/AA. We will also consider models that do assume an additive approach (rs7041: TT [reference] versus TG versus GG and rs4588: CC [reference] versus AC versus AA).

Covariates (from visit 2): Demographic factors: age (continuous, centered), age^2 (continuous, centered), sex (male; female), and race/center (Minnesota whites; Maryland whites; North Carolina whites; North Carolina blacks; Mississippi blacks).

Socioeconomic and lifestyle factors: education (<high school; high school, GED, vocational school; college, graduate or professional school), smoking (never; former; current), alcohol consumption (never; former; current), physical activity (continuous, centered), and vitamin D supplementation use (yes; no; more as a surrogate for health seeking behavior). We will consider supplements as nutritional products containing Vitamin D on the medication list from visit 2.

Cardiovascular disease related factors: body mass index (continuous), hypertension (yes; no; defined as diastolic blood pressure ≥90 mmHg or systolic blood pressure ≥140 mmHg or hypertension medication use), diabetes (yes; no; defined as fasting glucose ≥126 mg/dl or non-fasting glucose ≥200 mg/dl or self-reported physician diagnosis or diabetes medication use), total cholesterol and HDL cholesterol (continuous), prevalent stroke (yes; no; defined by standardized criteria and physician adjudication), and prevalent coronary heart disease (yes; no; defined by standardized criteria and physician adjudication).

Possible Effect Modifiers: Race and vitamin D binding protein SNPs (rs7041 and rs4588), pursuant to aims 3 and 4.

Outcome Ascertainments (Cognitive Tests): We will first look at the association of vitamin D with each of the individual 10 neuropsychological variables at ARIC Brain and ARIC NCS. For the primary analysis, these will be grouped into 4 domains representing general cognitive performance, memory, executive functioning, and language. In the subset attending both visits, we will also look at change in these 10 factors and 4 domains between ARIC Brain and ARIC NCS.

Data Analysis:

We will first look at the associations of vitamin D (measured at visit 2) with the three cognitive tests administered at visit 2 and each of the individual 10 neuropsychological tests at ARIC Brain (n~1100) and ARIC NCS (~n=6500). ARIC Brain is a smaller sample size but performed 12 years after vitamin D measurement. ARIC-NCS is a larger sample size, but nearly 20 years after the vitamin D measurement.

As we realize that this non-contemporary cross-sectional analysis described above has limitations, we will also focus on change in these neuropsychological factors between ARIC Brain and ARIC NCS. For the change analysis between ARIC Brain and ARIC NCS, briefly, we will use Generalized Estimating Equations (GEE) linear regression models with an unstructured correlation matrix (to account for within-person repeated cognitive test data) and robust variance to estimate the association between vitamin D and change in these 4 factor domains.

Models will be adjusted for age, age^2, sex, race/center, education, smoking, alcohol consumption, physical activity, vitamin D supplementation use, body mass index, hypertension, diabetes, prevalent stroke, and prevalent coronary heart disease. We will also consider further adjustment for biomarkers associated with 25(OH)D, including parathyroid hormone, calcium, and phosphate.

We will formally test for interaction by race and by vitamin D binding protein polymorphism genotype (rs7041 and rs4588). If there is any evidence for interaction by race or by polymorphism status, results will be presented stratified.

In accordance with the ARIC-NCS analysis working group recommendations, we will also perform sensitivity analyses to account for the significant attrition (dropout/death) in the cohort over the 20-year follow-up period. First, we will evaluate the extent of the vitamin D attrition. Then, if needed, we plan to use the MICE (multiple imputation by chained equations) methodology as developed by Rawlings and Sharrett, with sensitivity analysis including and excluding those who died in the imputation.

7.a. Will the data be used for non-CVD analysis in this manuscript? __X__ Yes ____ No

Yes – Cognition Outcome

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

Yes – we will look at vitamin D binding protein polymorphisms rs7041 and rs4588.

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)?

This proposal most closely overlaps with our previous proposal #2357 (Michos; Schneider) which also uses ARIC-NCS data to look vitamin D with 20 year cognitive decline, but that proposal focuses on a single test in each domain (DSST, WF, DWR). This current proposal is different in that we are utilizing the more extensive battery of neuropsychological tests available in ARIC Brain and ARIC NCS and doing a 4 domain factor analysis. We are also focusing on change between ARIC Brain and ARIC NCS in these factors.

This proposal also has similarity to previous proposal #2021 (Michos; Schneider), regarding vitamin D (measured at visit 3 in a subset ~1600) and cognitive decline through 2004-2006 (~10 year follow-up). This prior paper did not use ARIC-NCS data, nor did it use the full 12 neuropsychological tests.

Most of the writing committee is the same to ensure consistency of methods used.

This proposal also is similar to other proposals that investigate associations with 4 domain factor analysis in ARIC-NCS

Andreea Rawlings proposal #2033: Cognitive domains in elderly blacks and whites in the Atherosclerosis Risk in Communities Neurocognitive Study.

Alden Gross proposal #2215: Application of latent variable methods to the study of cognitive decline when tests change over time.

Retinopathy proposal – add number from ARIC dataset.

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)* _____ _____ _____)

Lutsey ARIC Ancillary Study number 2009.17
Michos ARIC Ancillary Study number 2010.01
Selvin ARIC Ancillary Study number 2009.16

*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.

Supplemental Table 1: Factor loadings for general and domain-specific factor analyses: Results from ARIC NCS (N=14,252)

Cognitive test	General cognitive performance		Memory	Language	Executive function	
	Factor loading (SE)		Factor loading (SE)	Factor loading (SE)	Factor loading (SE)	
	White	Black	White+Black	White+Black	White	Black
Delayed word recall	0.49 (0.01)		0.61 (0.01)			
Logical memory	0.58 (0.01)		0.70 (0.02)			
Incidental learning	0.58 (0.01)		0.63 (0.01)			
Trail Making Test, Part A	0.70 (0.01)	0.79 (0.01)			0.78 (0.01)	0.80 (0.02)
Trail Making Test, Part B	0.83 (0.01)				0.84 (0.01)	
Digit symbol substitution	0.79 (0.01)	0.86 (0.01)			0.74 (0.01)	
Semantic fluency	0.66 (0.01)			0.81 (0.01)		
Boston Naming	0.57 (0.01)	0.65 (0.01)		0.65 (0.01)		
Phonemic fluency	0.50 (0.01)	0.69 (0.01)		0.69 (0.01)		
Digit span backwards	0.47 (0.01)	0.56 (0.02)				

References:

[1] Robinson-Cohen C, Hoofnagle AN, Ix JH, Sachs MC, Tracy RP, Siscovick DS, et al. Racial differences in the association of serum 25-hydroxyvitamin d concentration with coronary heart disease events. *JAMA*. 2013;310:179-188

[2] Michos ED, Reis JP, Post WS, Lutsey PL, Gottesman RF, Mosley TH, Sharret AR, Melamed ML. 25(OH)D deficiency is associated with fatal stroke among whites but not blacks: The NHANES-III linked mortality files. *Nutrition* 2012; 28(4):367-71.

[3] Lutsey PL, Michos ED. Vitamin D, calcium, and atherosclerotic risk – evidence from serum levels and supplementation studies. *Curr Atheroscler Rep.* 2013; 15(1):293.

[4] Buell JS, Dawson-Hughes B. Vitamin d and neurocognitive dysfunction: Preventing "d"ecline? *Molecular aspects of medicine*. 2008;29:415-422

[5] Schneider ALC, Lutsey PL, Alonso A, Gottesman RF, Sharrett AR, Carson KA, Gross M, Post WS, Knopman DS, Mosley TH, Michos ED. Vitamin D and Cognitive Function and Dementia Risk in a Biracial Cohort: the ARIC Brain Ancillary Study. *Eur J Neurol.* 2014;21(9):1211-8, e69-70.

[6] Rawlings AM, Bandeed-Roch K, Sharrett AR et al. Cognitive domains in elderly blacks and whites in the Atherosclerosis Risk in Communities Neurocognitive Study. Paper in submission

[7] Gross AL, Power MC, Albert MS, et al. Application of latent variable methods to the study of cognitive decline when tests change over-time. Paper in preparation.

[8] Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, et al. Vitamin d-binding protein and vitamin d status of black americans and white americans. *N Engl J Med.* 2013;369:1991-2000

[9] Trummer O, Pilz S, Hoffmann MM, Winkelmann BR, Boehm BO, März W, Pieber TR, Obermayer-Pietsch B, Renner W. Vitamin D and mortality: a Mendelian randomization study. Clin Chem. 2013 May;59(5):793-7.