ARIC Manuscript Proposal #2020

PC Reviewed: 10/9/12  Status: A  Priority: 2
SC Reviewed: _________  Status: _____  Priority: ____

1.a. Full Title:

Vitamin D and subclinical cerebrovascular disease: an ARIC Brain MRI ancillary study

b. Abbreviated Title (Length 26 characters):

Vitamin D and cerebral WMH

2. Writing Group:

Writing group members:

<table>
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<th>Affiliation</th>
<th>Role</th>
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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 Vitamin D lab assays are currently being run from visit 3 serum. Anticipate lab work to be completed in early fall 2012 (by November 1). Plan to work on this manuscript late fall/early winter 2012/2013.

4. **Rationale:**

Low serum 25-hydroxyvitamin D [25(OH)D] levels are associated with cardiovascular disease (CVD) and its risk factors such as hypertension and diabetes. Emerging data suggest that vitamin D may be important for cognitive functioning and protective against neurovascular injury. Vitamin D is a neurosteroid, with vitamin D receptors widely expressed in the brain, both in neuronal and glial cells. Vitamin D may play a role in neuroprotection, perhaps through detoxification pathways, inhibition of inducible nitric oxide synthase, antioxidation/anti-inflammatory mechanisms, neuronal calcium regulation, or enhanced nerve conduction. Vitamin D may also prevent vascular injury through lowering blood pressure, inhibiting the renin-angiotensin-aldosterone system, and inhibiting atherogenesis.

White matter hyperintensities (WMH) and subclinical infarcts are commonly seen on brain MRIs of older adults. Because of their wide variability in prevalence among older adults, their association with cardiac disease, prior stroke, and CVD risk factors, WMH are believed to be at least partially preventable through identification and treatment of modifiable risk factors. WMH, even in the absence of obvious neurologic deficits, are associated with reduced functioning on cognitive testing and subjective mental decline. Thus the identification of novel and modifiable risk factors (such as potentially vitamin D deficiency) associated with WHM would have important clinical implications.

Prior work by Mosley in the ARIC Brain ancillary study of 1949 individuals (aged 55-72) without a history of clinical stroke or TIA, found both high grade WMHs and silent infarcts were independently associated with lower scores on all the cognitive tests performed. Furthermore, a subset (n=1134) underwent repeat brain MRI imaging 10 years later. Cumulative systolic blood pressure was found to be a strong predictor of WMH progression over followup. Worsening MRI status, including incident subclinical infarction, progression of WMH and ventricular enlargement, was significantly associated with 14-year cognitive decline.

Despite the association of vitamin D with clinical stroke, very little is known about the relationship of 25(OH)D with subclinical cerebrovascular pathology. Only one small cross-sectional study among elderly adults receiving home care services (n=318) found that lower vitamin D levels were associated with increased WMH volume and severity and the prevalence of large vessel infarcts. However, reverse causation may be one plausible explanation for the association found, as sicker individuals are less likely to be able to achieve physical activity outdoors and exposure to sunlight. No prior study has evaluated the prospective association of vitamin D with white matter changes or their progression in the brain, as newly proposed for this paper.
5. **Main Hypothesis/Study Questions:**

1: To determine the cross-sectional association of 25(OH)D levels measured at visit 3 with the prevalence of white matter hyperintensities (WMH) and subclinical cerebral infarcts measured by Brain MRI at ARIC visit 3 (1993-1994).

2: To determine the *prospective* association of 25(OH)D levels at visit 3 with progression (worsening) of WMH and incident subclinical infarcts measured by repeat brain MRI performed 10 years later (2004-2006).

**Hypothesis:** Low (compared with higher) 25(OH)D levels will be associated with increased subclinical cerebrovascular disease and its progression. Subclinical cerebrovascular disease, which includes WMH and subclinical infarcts, will be evaluated both cross-sectionally with 25(OH)D measurement (prevalent WMH and prevalent subclinical infarcts) and prospectively (greater WMH progression, and incident subclinical infarcts) independent of demographic and socioeconomic factors, vascular risk factors such as blood pressure and diabetes, and related biomarkers such as calcium, phosphate, and parathyroid hormone (PTH).

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).**

**Participants:** The ARIC Brain MRI ancillary study (R01 HL70825; PI Mosley) contains a subset of ARIC participants age ≥55 years from the Forsyth County and Jackson sites that were invited for a cerebral MRI and cognitive testing during the first two years of ARIC visit 3 (1993-1994) (n=1949, 60% women and 50% blacks). Inclusion/exclusion criteria for that ancillary study have previously been published. A subsequent ancillary grant (1R01NS072243-01; PI Michos) newly measured 25(OH)D, PTH, calcium, and phosphate levels from visit 3 stored blood from participants of ARIC Brain MRI ancillary study.

Participants with a history of stroke or TIA prior to visit 3 will be excluded.

<table>
<thead>
<tr>
<th>ARIC Study Timeline</th>
<th>ARIC Brain</th>
<th>ARIC Brain</th>
<th>ARIC-NCS</th>
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<tbody>
<tr>
<td>Exam</td>
<td>1  2  3  4</td>
<td>5</td>
<td></td>
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<tr>
<td>Followup year</td>
<td>0  3  6  9</td>
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<td>Number, N</td>
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<td>Age range, y</td>
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<td>48-67</td>
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<tr>
<td>Vascular risk factors</td>
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<td>X</td>
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Outcome ascertainment:
For the ARIC Brain MRI ancillary study, the cerebral MRI scanning protocol has been previously described. At ARIC visit 3, WMH severity was qualitatively scored from barely detectable white matter change (Grade 1) to extensive confluent changes (Grade 8). The absence of WMH was scored Grade 0, and those with changes worse than Grade 8 were scored as Grade 9. This rating scale from 0-9 was developed through the Cardiovascular Health Study (CHS).

At the 2004-2006 second ARIC Brain visit, in addition to qualitatively scoring WMH by visual inspection into grades 0-9, a semiautomated quantitative volumetric analysis was performed as previously described.

Since quantitative WMH were not available for visit 3 scans, the ARIC BrainAncillary Study investigators have imputed WMH volume scores for visit 3, as previously described. This provides 983 participants (49% black, 62% female, mean age of 72 years at the 2004-2006 visit) who completed 2 interpretable brain MRI scans. Of these, 4% of whites and 10% of blacks had WMH progression more than 2 grades. The average WMH change between the scans was 5.2 cm$^3$ (median 2.7 cm$^3$, SD 8.6 cm$^3$).

At both ARIC brain visits, brain MRIs were also scored separately for subclinical infarcts by size and location. Lacunes were defined as subcortical infarcts ≤20 mm in size. Incident infarcts will be considered as those seen on the second brain MRI among individuals with no infarcts on their first brain MRI.

[Repeat brain MRIs will be performed again in the ARIC-NCS study anticipated to be completed in 2013, which is estimated to include 547 survivors who had 2 previous brain MRI scans. As ARIC-NCS is not anticipated to be completed in time for this paper and given the reduced power with <550 having 3 scans, this paper proposal will only make use of the first 2 Brain MRI studies. The association of vitamin D with brain/cognitive markers measured in ARIC-NCS including brain MRI pathology will be the subject of another separate paper proposal.]

Statistical analysis:

1. For each analysis, 25(OH)D levels will be analyzed both as a continuous variable (including a spline analysis to allow for non-linearity) as well as in categorical thresholds of deficient (<15 ng/ml), insufficient (15-30 ng/ml), and sufficient (>30 ng/ml).
ng/ml). Prior studies have suggested a non-linear relationship of 25(OH)D with CVD risk, with increased risk primarily limited to the levels below <15 ng/ml).

2. The cross-sectional association of 25(OH)D levels with prevalent subclinical cerebral infarcts (>3 mm) at visit 3 (n=202, 13%) will be determined using multivariate logistic regression. Next, the cross-sectional association of 25(OH)D levels with high grade WMH will be determined using multivariate logistic regression. The distribution of WMH (measured qualitatively) is highly skewed and thus will also be dichotomized into no or mild grade abnormality (low grade) and moderate or high grade abnormality (high grade), as has previously been published. The “high grade” WMH group (n=161, 11%) is defined specifically as a grade 3 classification or higher. [Alternatively an ordinal logistic regression of increasing WMH groups will be considered, as has been used by CHS, despite the skewed character of the variable].

3. The association of 25(OH)D levels with progression of WMH and incident infarcts will be studied (participants with a clinical stroke prior to the second brain MRI will be excluded from analyses). Change in WMH volume will be calculated as the difference between estimated WMH volume at visit 3 from measured WMH at the 2004-2006 ARIC Brain MRI visit as done previously. Multivariable linear regression will be used to assess the relation of 25(OH)D levels with WMH change (with larger values indicating a larger increase in WMH between the 2 scans). Multivariable relative risk regression will estimate the effect of vitamin D on risk of being in the highest quintile (vs the lower 4 quintiles) of WMH volume change. Also the risk of advancing 1 category in WMH visual grade increase will be determined. Secondary analyses will also be performed after adjusting for baseline (visit 3) WMH. Multivariable relative risk regression will be used to evaluate the risk that vitamin D deficiency confers on incident subclinical infarct as detected by brain MRI (n=164 for incident cerebral infarct, n=131 for incident lacunar infarct measured in 2004-2006).

4. We may also evaluate a newly developed composite score, including both WMH and lacunes, in association with 25(OH)D levels.

5. Regression models will include both limited covariate adjustment for age, race, gender and comprehensive multivariate adjustment including traditional CVD risk factors, lifestyle factors, SES factors, and season of lab draw. Additional models will also include PTH, Calcium, Phosphate in the model to see if the association of 25(OH)D with cerebrovascular markers is still significant after taking into account these related biomarkers.

6. Interaction testing by race group will be performed

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 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?  
____ 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.cscce.unc.edu/ARIC/search.php

____X___ Yes  _______ No

Since 25(OH)D is being newly measured in the ARIC Brain ancillary study, there is no overlap with existing proposals.

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)?

Dr. Mosley who is the PI of the ARIC Brain Ancillary Study is a key investigator in my grant and a co-author on this proposal. Dr. Gottesman who led the paper about change in WMH in ARIC and develop the quantitative imputed scores for visit 3 is also a co-author. Drs. Mosley, Gottesman, and Sharrett have been involved in recent related ARIC Brain papers about cerebrovascular pathology. We will use similar methodology in this analysis. Dr. Laura Coker, a co-investigator in the ARIC Brain study, is also a co-author on this proposal.

There is another funded ARIC ancillary grant (PI Lutsey) that will measure 25(OH)D in the entire ARIC cohort to study the role of vitamin D with CVD outcomes. Dr Lutsey is a co-author on this proposal to ensure no overlap.

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

(1) Study PI: Erin Michos, Johns Hopkins, Funded grant NIH/NINDS: 1R01NS072243-01
11.b. If yes, is the proposal
   x___ A. primarily the result of an ancillary study (list number* _2010.0___)
   ___ 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.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.
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