1.a. Full Title: Serum fibroblast growth factor-23, phosphorus and risk of incident stroke: The Atherosclerosis Risk in Communities Study (ARIC)

b. Abbreviated Title (Length 26 characters): FGF23 & incident stroke


I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. _X_

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3. Timeline: Data analyses will begin immediately. Goal completion is Dec 2013.

4. Rationale:
Fibroblast growth factor 23 (FGF23) is a bone-derived endocrine hormone involved in the regulation of phosphorus homeostasis, vitamin D metabolism and bone mineralization. Its primary physiologic actions are to induce urinary phosphorus excretion, inhibit activation of calcitriol \([1,25(\text{OH})_2\text{D}]\), and suppress PTH synthesis\(^{1-3}\).
FGF23 levels are correlated inversely with renal function\textsuperscript{4, 5}. Both impaired kidney function\textsuperscript{6, 7} and low levels of serum vitamin D\textsuperscript{8, 9, 10-13} have been associated with greater stroke risk.

Most of the existing literature has focused on FGF23’s potential cardiac actions\textsuperscript{14-16}, however FGF23 may also be associated with risk of incident stroke. FGF23 was positively associated with risk of stroke in the Heart and Soul Study\textsuperscript{16}, and also among Cardiovascular Health Study participants with chronic kidney disease\textsuperscript{15}. FGF23 may increase stroke risk through either the CKD or vitamin D pathways. Furthermore, high circulating FGF23 has been associated with endothelial dysfunction\textsuperscript{17-19} and inflammation\textsuperscript{20, 21}; both of which have been associated with risk of incident stroke\textsuperscript{22-24}. Whether FGF23 is associated with atherosclerosis is controversial\textsuperscript{2, 3}. It is possible that phosphate excess, which is upstream to high FGF23, may induce vascular calcification and atherosclerosis, independent of FGF23\textsuperscript{2, 3}. Prior work has shown elevated serum phosphorus to be associated with vascular calcification\textsuperscript{25}, myocardial fibrosis\textsuperscript{26}, the development of left ventricular hypertrophy\textsuperscript{27}, and with greater risk of incident cardiovascular disease\textsuperscript{28-33}. However, research specifically looking at the association between phosphorus and stroke is limited.

Understanding of whether circulating levels of FGF23 and phosphorus are associated with stroke risk, particularly among those with normal kidney function, is incomplete. Hence we propose to evaluate these associations in the ARIC cohort.

5. Main Hypothesis/Study Questions:

We hypothesize that serum FGF23 and serum phosphorus will be positively associated with risk of incident stroke, independent of traditional CVD risk factors and kidney function.

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 from visit 2 through the 2010 events follow-up.

Inclusion/Exclusion

Participants with prevalent stroke at visit 2 will be excluded, as will those who are neither African American nor white, and African Americans from the MN and MD centers.

Variables

\textit{Exposures:} Serum FGF23 and serum phosphorus (measured in visit 2 serum)

\textit{Outcome:} Incident stroke. In secondary analyses we will look separately at ischemic, hemorrhagic and lacunar stroke.
**Confounders**: Age, race, sex, education, physical activity, smoking status, BMI, diabetes, LDL-C, HDL-C, triglycerides, and antihyperlipidemic medication use, systolic blood pressure, antihypertensive medication, CRP.

**Potential effect modifiers**: Age, race, sex, and eGFR (modeled as ≥90, 60-89, and ≤15 ml/min/1.73 m$^2$). eGFR will be calculated using both creatinine and cystatin-C$^{34}$.

**Additional variables of interest**: Serum phosphorus, parathyroid hormone, vitamin D, eGFR and FGF23 are physiologically interrelated. We intend to carefully model these biomarkers, and are cognizant of the difficulty in differentiating between confounding and mediation in situations such as this.

**Data analysis**

Visit 2 will serve as baseline for the current analysis. Visit 2 participant characteristics will be described using means and proportions stratified by levels of the exposures.

Cox proportional hazards regression will be used to explore associations between FGF23 and risk of incident stroke. We will use cubic splines to visually depict the associations, and aid in selecting the most appropriate exposure representation. Our first model will adjust for age, sex, race and center. Model 2 will additionally adjust for education, physical activity, smoking status and BMI. Model 3 will further adjust for prevalent diabetes, systolic BP, hypertension medication use, lipid medication use, LDL-C, HDL-C and CRP. Additional models will also adjust for eGFR, PTH, vitamin D, and where FGF23 is the main exposure, serum phosphorus. Cross-product terms will be used to evaluate whether age, race, sex, and/or eGFR modify associations between FGF23 and risk of incident stroke. Stratified results will be presented, as appropriate. Secondary analyses will look separately at the association of FGF23 to risk of incident ischemic stroke, hemorrhagic stroke, and lacunar stroke. Sensitivity analyses will also be conducted where we exclude participants with prevalent HF, CHD or AF, and censor participants upon development of HF, CHD or AF.

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  ____ X  ____ 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.cscu.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)?


#2088: Association of fibroblast growth factor 23 (FGF-23) with incidence of atrial fibrillation: the ARIC study Alvaro Alonso, Jeffrey Misialek, John Eckfeldt, Liz Selvin, Josef Coresh, Lin Y. Chen, Elsayed Soliman, Sunil Agarwal, Pamela L. Lutsey


#2143: Association of fibroblast growth factor-23 levels with risk and progression of chronic kidney disease: the Atherosclerosis Risk in Communities (ARIC) Study Casey M. Rebholz, Morgan Grams, Josef Coresh, Elizabeth Selvin, Pamela L. Lutsey


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

2009.17 (Lutsey PI)  
- “Serum vitamin D and cardiovascular disease risk in the biethnic ARIC cohort”
2009.16 (Selvin PI)
- “Short-term markers of glycemia and long-term outcomes”
- Numerous biomarkers which may be confounders and/or effect modifiers in the present analysis are being measured as part of this grant (e.g. CysC, CRP).

  ___ 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/

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.

References

8. Michos ED, Reis JP, Post W, Lutsey PL, Gottesman RF, Mosley TH, Sharrett AR, Melamed ML. Vitamin d deficiency is associated with increased risk of death from cerebrovascular disease among whites but not blacks: The nhanes-iii linked mortality files. Abstract Submitted to: American Heart Association Scientific Sessions. 2010


