1.a. Full Title: Metabolomics and Cognitive Impairment: The Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): Metabolomics and Dementia

2. Writing Group:
   Writing group members: Jan Bressler, Bing Yu, Thomas H. Mosley, David S. Knopman, Rebecca F. Gottesman, and Eric Boerwinkle

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

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4. Rationale:
Alzheimer’s disease (AD) is the most common form of dementia and is characterized by significant impairment in memory, behavioral changes, and gradual loss of autonomy. There is currently no known cure or preventive intervention. Because there is a long latent period prior to diagnosis identification of blood-based biomarkers that could help to identify those at risk in the early preclinical phase is a priority. Mapstone et al. have recently reported that a set of ten lipids identified in a metabolomics screen in peripheral blood could be used with 90% accuracy to predict conversion from normal cognitive status to amnestic mild cognitive impairment (MCI) or AD over a 2-3-year period in adults 70 years or older. Several other investigators have also found significant alterations in metabolic profiles in comparisons of patients with MCI and AD to cognitively normal subjects. The goal of this study is to determine whether metabolites measured in serum in middle-aged African-American and European-American adults are associated with dementia and mild cognitive impairment (MCI) in the ARIC study.

5. Main Hypothesis/Study Questions:

This proposed research will use a longitudinal design. The following hypotheses will be examined:

1. Dementia is associated with metabolites detectable in the current metabolomic profile.
2. MCI is associated with metabolites detectable in the current metabolomics profile.

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 and Sample

This is a longitudinal study that consists of African-American and European-American ARIC participants at the visit 5/NCS examinations. Metabolomics data was obtained at visit 1. Dementia and MCI case status will be assessed using a derived variable provided by the ARIC Coordinating Center. All analyses will be performed separately for dementia and MCI by race.

Exclusions:
- Missing outcome or covariates information

Outcome Variables:
- Dementia at visit 5
- MCI at visit 5

Covariates:
- Age
- Sex
- The kidney filters all metabolites from the blood. Molecules of <10,000 Da molecular weight are freely filtered by the kidney and subsequently metabolized (reabsorbed, catabolized and/or secreted). eGFR at visit 1 at the time the metabolites were measured will be calculated based on serum creatinine using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation as follows: $eGFR_{\text{CKD-EPI}} = 141 \times \min\left(\frac{\text{Standard Serum Creatinine [mg/dL]}}{\kappa}, 1\right) a \times \max\left(1, \frac{\text{Standard Serum Creatinine [mg/dL]}}{\kappa}\right)$ (minimum of Standard Serum Creatinine [mg/dL]/κ or 1) $a \times$ (maximum of Standard Serum Creatinine [mg/dL]/κ or 1)


Serum Creatinine \([\text{mg/dL}] / \kappa \text{ or } 1\) \(1.209 \times 0.993^{\text{age}} \times (1.018 \text{ if female}) \times (1.159 \text{ if black}),\)
where \(\kappa \) is 0.7 if female and 0.9 if male; \(\alpha \) is -0.329 if female and -0.411 if male.

**Metabolomics data:**
Based on both practical and theoretical considerations, we will limit the analysis to those metabolites with acceptable reliability (\(r > 0.6\)), no obvious batch effect, low missing rate (< 25% in both batches) and shared between African Americans and European Americans. It is estimated that there are to be 245 named metabolites that satisfy this criteria. Metabolites with missing/below detection limit in <25% of the samples will be analyzed as continuous variables, assigning the lowest detected value for that metabolite to those with missing/below detection limit.

**Statistical Methods:**
Logistic regression models will be applied for each metabolite to estimate its association with either dementia or MCI. In secondary analyses, regression models will be adjusted for vascular risk factors at visit 5 (diabetes, hypertension, low density lipoprotein cholesterol, current smoking) and APOE genotype in addition to the covariates listed above. Statistical significance for the metabolomic data will be pre-specified with an experiment-wise \(\alpha = 0.05\) (2 tailed) and a modified Bonferroni procedure will be used to consider the correlations among metabolites.

All of the analyses described for Aims 1 and 2 will be performed by Jan Bressler and Bing Yu under the supervision of Eric Boerwinkle; a signed data distribution agreement has been completed.

**Limitations:**
The limitations of the current study include assessment of the metabolomics data at visit 1 while cognitive impairment was assessed at visit 5. In support of this approach, Yan Zheng et al. have previously reported detection of novel biomarkers and pathways significantly associated with incident hypertension\(^{13}\) and incident heart failure\(^{14}\) during a 10-year or 20-year follow-up period, respectively, using the same dataset. A second caveat is that the metabolomic profile is available for a subset of randomly selected ARIC participants at visit 1 (\(n = 1,977\) African-Americans; \(n = 1,533\) European-Americans) who may differ in demographic and clinical characteristics from those individuals for whom metabolomics data is not available.

**References:**


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? _____ 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”? ___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 list under the Study Members Area of the web site at: [http://www.cscs.unc.edu/ARIC/search.php](http://www.cscs.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)?

MS#1847 Zheng Y et al. Role of the Human Metabolome in Incident Heart Failure Etiology among African Americans in the Atherosclerosis Risk in Communities (ARIC) Study

MS#1853 Yu B et al. Genome-Wide Association Study of Heart Failure-related Human Metabolite Profiles among African Americans in the Atherosclerosis Risk in Communities (ARIC) Study.


MS#1918 Zheng Y et al. Associations of the Human Metabolome with Blood Pressure, Prevalent and Incident Hypertension among African-Americans in the Atherosclerosis Risk in Communities (ARIC) Study.

MS# 2120 Knopman D et al. Prevalence of Mild Cognitive Impairment and Dementia and Their Relationship to Diabetes and Hypertension in ARIC.

MS#2354 Alonso A et al. Metabolomics and Incident Atrial Fibrillation in African-Americans: the ARIC study.


MS# Li D et al. Plasma phospholipids and prevalence of mild cognitive impairment/dementia in the ARIC Neurocognitive Study.

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* 2008.16; Metabolomics and Heart Failure: A Novel Approach to Biomarker Discovery

_____ 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.csc.c.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.

13. Per Data Use Agreement Addendum for the Use of Linked ARIC CMS Data, approved manuscripts using linked ARIC 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.