ARIC Manuscript Proposal #3509

1.a. Full Title: Proteomics of apolipoprotein E genetic polymorphisms: The Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): Proteomics of APOE genotype

2. Writing Group: Pamela L Lutsey
   Writing group members: Confirmed, Weihong Tang, Jim Pankow, Aaron Folsom, Faye Norby, Ryan Demmer, Keenan Walker. Also invited: Joe Coresh, Jeannette Simino, Christie Ballantyne, Eric Boerwinkle. We welcome additional nominations, particularly of others who have been instrumental in the proteomics data.

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

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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: Analyses will begin immediately; pen draft expected in ~6 mos.

4. Rationale:
   Apolipoprotein E (ApoE) is a major cholesterol carrier that supports lipid transport and injury repair in the brain. Common ApoE polymorphisms – which are a combination of two
genetic variants (rs429358 and rs7412, both being missense variants) on chromosome 19 – have emerged as a poignant risk factor for both cardiovascular and neurodegenerative conditions. The three common alleles coded by the ApoE gene differ by only a single amino acid interchange, and occur at different frequencies in humans (ε2, 5–10%; ε3, 65–70%; and ε4, 15–20%). They give rise to three homozygous (ε2/ε2, ε3/ε3, and ε4/ε4) and three heterozygous (ε3/ε2, ε4/ε2, and ε4/ε3) phenotypes. Liver hepatocytes synthesize ~75% of the body’s plasma apoE, however these molecules cannot cross the blood brain barrier. The organ with the next greatest production is the brain, were it is produced primarily by astrocytes, but also by oligodendrocytes, microglia and neurons (particularly injured or stressed neurons).

Mendelian randomization analyses have suggested that APOE ε4 is casually associated with dementia. In a meta-analysis of clinical and autopsy-based studies, compared with individuals with an ε3/ε3 genotype, risk of Alzheimer’s disease was greatest among individuals with two copies ε4 allele (ε4/ε4, OR 14.9) and intermediate among those with one copy (ε2/ε4, OR 2.6; ε3/ε4, OR 3.2). Presence of the ε2 allele of APOE was associated with lower risk of Alzheimer’s disease [APOE ε2/ε2 (OR 0.6) or ε2/ε3 (OR 0.6)] relative to ε3/ε3. APOE has an important role in amyloid beta (Aβ) metabolism. ApoE isoforms differentially regulate Aβ aggregation and clearance in the brain, and have distinct functions in regulating brain lipid transport, glucose metabolism, neuronal signaling, neuroinflammation, and mitochondrial function. The isoforms strongly affect deposition of Aβ to form senile plaques and cause cerebral amyloid angiopathy; both of which are hallmarks Alzheimer’s disease.

Despite being now more widely recognized as a dementia risk factor, ApoE was first discovered in the context of lipid metabolism and cardiovascular disease risk. ApoE plays a major role in regulating cholesterol homeostasis by mediating uptake of very low density cholesterol (VLDL), intermediate density lipoproteins and chylomicron remnants. Apo ε4 bind preferentially to large (30-80 nm) triglyceride-rich VLDL, whereas ApoE ε3 and Apo ε2 preferentially bind to small (9-16 nm) high density lipoprotein cholesterol (HDL) particles. Apo ε4 is associated with elevated LDL concentrations, and greater risk of atherosclerosis, whereas Apo ε2 binds defectively to the LDL receptor and can precipitate type III hypercholesteremia. Concentrations of plasma ApoE and, generally also, high-density lipoprotein cholesterol (HDL-C) decrease in a parametric fashion across the six APOE genotypes (ε2/ε2 > ε2/ε3 > ε2/ε4 > ε3/ε3 > ε3/ε4 > ε4/ε4), and the reverse is true for levels of low-density lipoprotein cholesterol (LDL-C). A similar stepwise pattern is seen in the risk for coronary artery disease and myocardial infarction, where APOε2 carriers have the least risk, APOε4 carriers have the greatest risk, and APOε3 carriers fall in the middle. In a prior analysis of ARIC data, the hazard ratio for CHD was 1.09 (1.00-1.19) for ε4 carriers (ε3/ε4 and ε4/ε4) and 0.88 (95% CI: 0.77-0.99) for ε2 carriers (ε2/ε2 and ε2/ε3) and compared with homozygous ε3/ε3 individuals after age and sex adjustment. These associations were explained by the inclusion of serum lipid concentrations in the models.

While much is known about the pathophysiology of how APOE alleles influence cardiometabolic and neurodegenerative disorders, it is possible that additional pathways remain to be elucidated. Discovery of new pathways could lead to novel therapeutic and preventive approaches. The ARIC SomaScan data provide a unique and unprecedented resource with which to identify new pathways through which APOE isoforms influence human health and disease.
5. Main Hypothesis/Study Questions:

Hypothesis: APOE is a pleiotropic genetic variant that will demonstrate significant associations with numerous proteins – both known and novel – in the plasma proteome.

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).

Design: Cross-sectional. Visit 3 will be used for the primary analysis. Visit 5 will serve as a nuanced replication sample. Visit 3 was selected for the primary analysis due to the larger N, less medication use, less kidney impairment, fewer comorbidities.

Inclusion/Exclusion: We will exclude participants who (a) are not black or white, blacks from MD and MN; (b) do not have SOMAscan data at visit 3 or failed QC; or (c) do not have APOE genotypes.

Exposure: For the primary analysis we will classify APOE isoforms as e4/e4 (~3% of ARIC population), e2/e4 or e3/4 (~18%), other (~69%). To maximize power, at least preliminarily we will model this continuously (1 degree of freedom test). We may also explore alternate modeling (e.g. 2 degree of freedom test, or the isoforms as \( \varepsilon_2/\varepsilon_2 > \varepsilon_2/\varepsilon_3 > \varepsilon_2/\varepsilon_4 > \varepsilon_3/\varepsilon_3 > \varepsilon_3/\varepsilon_4 > \varepsilon_4/\varepsilon_4 \)) in sensitivity analyses.

Outcome: Natural log of SomaScan protein levels. Non-human proteins and proteins with unacceptable QC will be removed.

Data analysis: Multiple linear regression, with protein level as the dependent variable and APOE genotype as the main independent variable. Primary analysis will combine both whites and blacks. Covariates will include age at visit 3, sex, race, principal components of ancestry, and eGFR. The false discovery rate will be applied to determine the threshold for statistical significance. For significant proteins, we will evaluate whether the strength of association differs by race or sex. As a sensitivity analysis, we will repeat the analyses at visit 5.

In secondary analyses we may also explore associations between or top hits and SomaScan plasma ApoE. However, we have reservations about this analysis since on the SomaScan panel plasma ApoE has a reliability coefficient of only 0.6.

To complement our findings, we will use Ingenuity Pathway Analysis (IPA) to identify pathways of potential interest.

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
8.a. Will the DNA data be used in this manuscript? _X__ Yes    ____ 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 lists under the Study Members Area of the web site at: http://www.cscc.unc.edu/aric/mantrack/maintain/search/dtSearch.html

____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)?

#1912: APOE modulates the relationship among triglycerides, cholesterol, and CHD through pleiotropy and gene-gene interactions. (Taylor Maxwell 1st author, Eric Boerwinkle senior)

#3281: APOEε4 and time vs risk to dementia (Danielle Powell 1st)

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* ___ AS2017.27 ___)

___ 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 https://www2.cscc.unc.edu/aric/approved-ancillary-studies

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