1.a. **Full Title**: 20-Hydroxyeicosatetraenoic Acid and Cognitive Impairment in Aging and Hypertension

b. **Abbreviated Title (Length 26 characters)**: 20-HETE and Dementia

2. **Writing Group**:
   Writing group members: Fan Fan, Jeannette Simino, Eric Boerwinkle, Myriam Fornage, David Knopman, Alexander Auchus, Tom Mosley, Richard Roman

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

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3. **Timeline**: Manuscript: March 2016
4. Rationale:

Alzheimer’s disease (AD) is the most commonly diagnosed cause of cognitive impairment among the elderly, however, at autopsy most AD cases also have vascular lesions which may affect AD onset and progression in addition to independently causing cognitive impairment and dementia. The myogenic response and autoregulation of cerebral blood flow (CBF) is often impaired in aging individuals, especially in those with hypertension. This impairment may contribute to age-related cerebral vascular disease including vascular cognitive impairment (VCI) and AD. Autoregulation of CBF is primarily mediated by myogenic constriction of cerebral arteries in response to elevations of cerebral perfusion pressure (CPP). It is a critical homeostatic mechanism that maintains constant oxygen delivery to the brain despite fluctuations in CPP. It protects the brain from capillary damage, BBB leakage and edema following elevations in CPP. However, very little is known about the causes and mechanisms involved due to the lack of a genetic animal model exhibiting an impaired myogenic response.

20-HETE is a metabolite of arachidonic acid (AA) catalyzed by enzymes of CYP4A and 4F families. It is a potent vasoconstrictor and plays a critical role in the myogenic response and autoregulation of CBF. We recently reported that a genetic deficiency in the formation of 20-HETE was reduced in the cerebral vasculature of Dahl salt sensitive (SS) rats and impaired the myogenic response and autoregulation of CBF that was associated with increased blood brain barrier (BBB) permeability. Transfer of chromosome 5 containing the CYP4A genes from normal Brown Norway (BN) rats (SS.5BN) or introduction of a wild type CYP4A1 onto the SS genetic background (SS. CYP4A1) restores the autoregulation of CBF and reduced BBB leakage. Capillary pressure was higher and capillary density was reduced in SS vs. SS.5BN rats, and SS rats exhibited characteristics of VCI such as BBB leakage, vascular remodeling, neurodegeneration, and learning and memory impairments with aging and hypertension. Human studies have linked sequence variants of CYP4A11 and CYP4F2 to the development of hypertension and stroke; however, no studies have been done to determine if sequence variants in these genes are linked to cerebral cognitive impairments especially in aging and hypertensive patients. Furthermore, our animal studies indicate that these effects occur with two copies of the aberrant allele, necessitating the use of non-additive models.

5. Main Hypothesis/Study Questions:

Our overall goal is to determine if two genes (CYP4F2 and CYP4A11) involved in the myogenic response and autoregulation of cerebral blood flow in hypertensive rats are associated with neurocognitive, cerebrovascular, or neuroimaging traits in humans. Our specific aims are as follows:

a. To identify common CYP4F2 and CYP4A11 variants associated with cognitive impairment (dementia/MCI) or neuroimaging traits (hippocampal volume, white matter hyperintensity volume, or infarcts) in ARIC African and European Americans
b. To determine whether rare functional variants in \textit{CYP4F2} and \textit{CYP4A11} are associated with cognitive impairment (dementia/MCI) or neuroimaging traits (hippocampal volume, white matter hyperintensity volume, or infarcts) by gene-based tests in ARIC African and European Americans

In future work, the mechanisms for any associated cognitive or MRI-defined traits will be explored using the Dahl SS rat model of aging and hypertension.

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).

\textit{Study Design:}
Cross-sectional candidate gene study of the ARIC V5/NCS participants

\textit{Exclusion Criteria:}
- Participants missing outcomes, covariates, or exome sequence
- Participants of non-African and non-European descent
- Individuals who did not provide consent for DNA use
- African Americans (AAs) from the suburban Minneapolis, Minnesota field center
- European Americans (EAs) from the Jackson, Mississippi field center
- Individuals with hippocampal volumes of 0 will be excluded from the analysis of that outcome only

\textit{Outcomes (at visit 5 or NCS):}

\textit{Previously associated outcomes (Confirm these associations in our samples):}
- Stroke (defined through the most recent surveillance data)
- Blood pressure (SBP, DBP, MAP, PP, Hypertension): SBP and PP were log-transformed after medication adjustments

\textit{Hypothesized associations (Primary focus of our analyses):}
- Cognitive impairment (algorithmic diagnosis dichotomized into MCI/dementia vs normal)
- Hippocampal volume
- Volume of white matter hyperintensities (log2-transformed)
- Infarct on MRI (Yes/No)

\textit{Covariates:}
All outcomes will be adjusted for age (in years) at V5/NCS, gender, field center, and Eigenstrat-derived principal components (to control for population substructure). In addition, specific traits will be adjusted as follows:
• Blood pressure traits will include age-squared and body-mass-index recorded at the fifth examination
• Cognitive impairment will include the apolipoprotein E (APOE) ε4 genotype ascertained from TaqMan assays
• Hippocampal and white matter intensity volume will include estimated total intracranial volume and the APOE ε4 genotype

Given the low frequency of APOE ε4 homozygotes in EA participants, we will use an indicator of ε4 allele presence (Yes/No) in the EA-specific models. For AAs, we will use two dichotomous variables indicating heterozygosity and homozygosity of the APOE ε4 allele.

**Exome Sequence:**

Our analysis sample will include 3,649 (1,116 AAs, 2,533 EAs) participants with Freeze 4 whole exome sequence and ARIC fifth visit outcomes (cognitive impairment, blood pressure, and stroke). Of these, 1,185 participants (396 AAs, 789 EAs) have MRI outcomes (brain volumes and infarct assessment). For the single variant tests, we will test the following nonsynonymous and synonymous SNPs:

*CYP4A11:*
- rs1126742
- rs1126743

*CYP4F2:*
- rs2108622
- rs2074900
- rs3093153
- rs3093136 (AAs only)
- rs8110714 (AAs only)
- rs8100960 (AAs only)
- rs3093114
- rs3093106
- rs114099324 (AAs only)
- rs3093105

There are 72 and 106 annotated variants in *CYP4A11* and *CYP4F2*, respectively. We will restrict the gene-based tests to the nonsynonymous, splicing, stopgain, stoploss, and frameshift variants.

This exome sequence data was quality-controlled as part of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium. A full annotation file and description of the methods is available.
**Statistical Methods:**

Under the premise that both hypertension and aging can inflict cerebrovascular changes, we will include all participants from V5/NCS. A secondary analysis of cognitive traits will be restricted to hypertensives only. We will add 10 mmHg to the observed SBP and 5 mmHg to the observed DBP of individuals prescribed antihypertensives. MAP and PP will be calculated from the medication-adjusted SBP and DBP values. Individuals with BMI or blood pressure values exceeding four standard deviations from the mean will be excluded from all blood pressure analyses. The medication-adjusted SBP and PP were skewed with excess kurtosis, thus we will apply a log-transform. Similarly, the distributions of the brain volumes were scrutinized for outliers and normality, thus the white matter hyperintensity volume will be log2-transformed.

For the single variant tests, we will use the program ProbABEL to fit ethnic-specific linear and logistic models to the non-binary and binary traits, respectively, while adjusting for the covariates. We will fit additive, dominant/recessive, and genotypic (2df) models. For the gene-based tests, we will use seqMeta version 1.5 to conduct ethnic-specific tests while adjusting for the covariates. Sequence Kernel Association Tests (SKAT) and T5 gene-based tests will be conducted on nonsynonymous, splicing, stopgain, stoploss, or frameshift autosomal variants with MAF ≤ 0.05. For both the single-variant and gene-based tests, we will conduct a meta-analysis of the African and European American results and apply a Bonferroni correction for the total number of statistical tests conducted.

**Limitations:**

- We have a small sample by genetic study standards. Using exome sequence exacerbates the problem, but unfortunately many of these SNPs are not available in our 1000G GWAS data. For example, nonsynonymous SNP rs1126742 (one of only two SNPs tested in CYP4A11) is not available in GWAS data from either ethnicity. For SNPs available and of sufficient quality in GWAS, we will repeat the analyses using this larger sample which will also allow an assessment of the importance of NCS sampling weights when analyzing MRI traits.

- There may be too few homozygotes to fit recessive and genotypic models for some of the SNPs. In those cases, we will restrict the analyses to additive models.

- For the MRI measures, we do not have a representative sample. The aforementioned sensitivity analysis using the larger sample with GWAS SNPs will provide insight into the severity of the bias.

- Using pure vascular MCI/dementia as an outcome seems ideal, however there are too few participants with this diagnosis and exome sequence. However, we can collapse any etiology that contains CVD and compare it to those with MCI/dementia due to non-vascular causes or no MCI/dementia at all.
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?  __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/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)?

*Genetic studies of cognitive phenotypes:*

**Manuscript #1120:** EPHX2 polymorphisms, MRI abnormalities, and cognitive decline in African Americans (Lead author: Myriam Fornage)

**Manuscript #1363:** PCSK9 sequence variation and cognitive decline (Lead author: Jan Bressler)

**Manuscript #1393:** Candidate gene associations, epistatic interactions, and pleiotropy in MRI-defined structural brain injury: the Atherosclerosis Risk in Communities Study (Lead author: Myriam Fornage)

**Manuscript #1394:** Genome-wide association study of MRI-defined covert infarcts and white matter lesion in the CHARGE consortium (Lead author: Myriam Fornage)

**Manuscript #1703:** Identification of candidate genes associated with cardiovascular disease (CVD) that predict cognitive change in mid-life: The Atherosclerosis Risk in Communities (ARIC) Study (Lead author: Jan Bressler)

**Manuscript #1704:** Genetic variants identified in genome-wide association studies of dementia and cognitive change in middle age: The Atherosclerosis Risk in Communities (ARIC) Study (Lead author: Jan Bressler)

**Manuscript #1705:** Sequence Variation in FTO and Cognitive Decline: The Atherosclerosis Risk in Communities Study (Lead author: Jan Bressler)

**Manuscript #1771:** Cognitive, vascular risk factor and APOE genotype predictors of hippocampal volume (Lead author: David Knopman)
Manuscript #1928: Genome-wide methylation analyses of cardiovascular disease (CVD) and its risk factors (Lead author: Jan Bressler)
Manuscript #2079: Genome-wide studies of verbal declarative memory: The CHARGE consortium
Manuscript #2080: Genome-wide association studies for executive function and processing speed indicate a role for genes in neurotransmission (Lead author: Jan Bressler)
Manuscript #2337: DNA methylation-derived age predicts changes in brain morphology and cognitive decline (Lead author: Myriam Fornage)
Manuscript #2451: Genome-wide association study of fine motor function in the CHARGE consortium (Lead author: Jan Bressler)
Manuscript #2502: 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 Study (Lead author: Erin D. Michos)
Manuscript #2564: Variation in ethanol-metabolizing genes modifies the relationship between ethanol intake and cognitive decline: The ARIC Neurocognitive Study (Lead author: Shelly-Ann M Love)

Investigation of the connection between blood pressure/stroke and cognitive traits:

Manuscript #388: Association of cognitive function with hypertension, its treatment and control-The ARIC Study (Lead author: Duanping Liao)
Manuscript #672: Changes in cognitive test scores in the ARIC cohort over a 6-year period (Visit 2 to Visit 4) and their correlation with vascular risk factors (Lead author: David Knopman)
Manuscript #1010: Omega-3 fatty acids, hypertension and risk of cognitive decline among older adults: The Atherosclerosis Risk in Communities (ARIC) study (Lead author: May A. Beydoun)
Manuscript #1121: Cognitive change over 12 years and its relationship to cardiovascular risk factors ARIC MR Study (Lead author: David Knopman)
Manuscript #1387: Temporal changes in blood pressure and cerebral white matter lesions in a biethnic sample: The ARIC MRI study (Lead author: Rebecca F. Gottesman)
Manuscript #1973: Cardiovascular exposures, cognitive decline and depression in whites and blacks (Lead author: Adina Zeki Al Hazzouri)
Manuscript #2120: Prevalence of mild cognitive impairment and dementia and their relationship to diabetes and hypertension in ARIC (Lead author: David Knopman)
Manuscript #2120B: Mid-life vascular risk factors for mild cognitive impairment in the ARIC NCS Study (Lead author: David Knopman)
Manuscript #2175: Midlife blood pressure and 20-year cognitive change: The ARIC Neurocognitive Study (Lead author: Rebecca Gottesman)
Manuscript #2351: Association of blood pressure with neurodegenerative and cerebrovascular changes on brain MRI (Lead author: Melinda Power)
Manuscript #2358: Association of posture-dependent changes in blood pressure with cerebral vascular lesions: the ARIC Neurocognitive Study (Lead author: Anna Poon)
Manuscript #2483: Brain Health in African Americans: The ARIC experience (Lead author: Rebecca F. Gottesman)
Manuscript #2539: Mid-life long-term blood pressure variability and late-life cognitive decline: The ARIC Neurocognitive Study (Lead author: Yuichiro Yano)

Manuscript #2549: Stroke risk scores and white matter hyperintensity progression: The Atherosclerosis Risk in Communities Study (Lead: Rebecca Gottesman)

Manuscript #2551: Midlife and late life vascular risk factors and white matter integrity assessed using diffusion tensor imaging: the ARIC-NCS study (Lead author: Melinda Power)

Manuscript #2591: Association of ICAD with dementia and mild cognitive impairment: the ARIC-Neurocognitive Study (Lead author: M Fareed K. Suri)

Genetic of blood pressure and stroke:

Manuscript #716: Angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism and angiotensigen (AGT) G-6A polymorphism predict stoke case status (Lead: Eric Boerwinkle)

Manuscript #732: apolipoprotein E (apoE) and lipoprotein lipase (LPL) Asn291Ser and Ser447Ter polymorphisms and risk of subclinical and clinical stroke (Lead author: Eric Boerwinkle)

Manuscript #1140: Association of a Novel Prothrombin (FII) Variant (PT20209) in African Americans with Stroke and Myocardial Infarct (Lead author: Lisa Vincent)

Manuscript #1390: Genome-wide association study of incident stroke in the CHARGE consortium (Lead author: Myriam Fornage)

Manuscript #1408: CHARGE GWAS for BP (SBP and DBP) at first visit (Lead author: Georg B. Ehret)

Manuscript #1412: GWAS for longitudinal blood pressure levels (Lead author: Georg B. Ehret)

Manuscript #1430: Genotype-by-smoking and the risk of atherosclerosis and its clinical sequelae: the ARIC Study (Lead author: Christy Avery)

Manuscript #1484: A gene-environment interaction approach to genome-wide association analysis of blood pressure in the ARIC Study: Gene-age interactions in European Americans (Lead author: Gang Shi)

Manuscript #1509: ICBP GWAS for BP (SBP and DBP) at first visit (Lead author: Georg B. Ehret)

Manuscript #1510: Gene-body mass index interactions influencing blood pressure in Caucasians (Lead author: Yan V. Sun)

Manuscript #1513: Genome-wide association study of blood pressure using genotype-by-smoking and genotype-by-alcohol intake interactions: the ARIC Study (Lead author: Nora Franceschini)

Manuscript #1542: Rare variants of CHARGE GWAS for BP (SBP and DBP) at first visit (Lead author: Tao Feng)

Manuscript #1548: CHARGE GWAS for BP (SBP, DBP, HTN) at first visit: sex specific analysis (Lead author: Georg B. Ehret)

Manuscript #1549: ICBP GWAS for BP (SBP, DBP, HTN) top findings: rare variant analysis in ARIC (Lead author: Georg B. Ehret)
Manuscript #1650: Generalization of genome-wide association study findings for blood pressure to orthostatic hypotension traits in individuals of European and African ancestry (Lead author: Nora Franceschini)

Manuscript #1763: CHARGE for BP (SBP, DBP, MAP, PP, HTN): CHARGE-S sequencing (Lead author: Aravinda Chakravarti)

Manuscript #1843: Effects of Rare and Common Blood Pressure Gene Variants on Essential Hypertension: Results from the FBPP, CLUE, and ARIC studies (Lead author: Khanh-Dung H. Nguyen)

Manuscript #1851: Genetic association of pulse pressure in the multi-ethnic cohorts of the PAGE consortium (Lead author: Petra Buzkova)

Manuscript # 1868: Discovery and fine mapping of blood pressure loci to African Americans and Hispanic individuals using the MetaboChip array: The PAGE Study (Lead author: Nora Franceschini)

Manuscript #1870: Antihypertensive drug-gene interactions and cardiovascular events (Lead author: Christy L. Avery)

Manuscript #1908: Replication of a method for estimating the impact of the parent of origin for genetic association (Lead author: George Ehret)

Manuscript #1995: Genome-wide association study of stroke risk in patients with atrial fibrillation: the CHARGE consortium (Lead author: Alvaro Alonso)

Manuscript #2068: Exome chip and exome sequencing analyses for BP phenotypes (Lead author: Aravinda Chakravarti)

Manuscript #2124: Candidate Gene Association Resource (CARe) Project Database Application (Project coordinator: James B Meigs)

Manuscript #2203: Chronic inflammation and race-ethnic disparities in ischemic stroke: the ARIC study (Lead author: Cheryl Bushness)

Manuscript #2332: Relationship loci (rQTL) and variance heterogeneity loci (vQTL) among blood pressure measured, incident CHD and loci that interact with them (Lead author: Taylor J. Maxwell)

Manuscript #2438: Drug*Gene GWAS of blood pressure response to anti-hypertensives in the CHARGE Consortium (Lead author: Eric A. Whitsel)

Manuscript #2464: A multi-ethnic study of gene-lifestyle interactions in cardiovascular traits: the CHARGE consortium (Lead author: Alanna C. Morrison)

Manuscript #2499: Genomewide association study of blood pressure trajectories from midlife to older age: the Atherosclerosis Risk in Communities (ARIC) Study (Lead author: Poojitha Balakrishnan)

Manuscript #2666: The influence of sodium and potassium in the context of genetic variants associated with blood pressure in the Population Architecture using Genomics and Epidemiology (PAGE) study (Lead author: Cynthia Bell)

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*  2008.06)
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.cscu.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. We agree to complete the manuscript in less than three years.

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.cscu.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. Yes, we agree to abide by the public access policy.

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.