ARIC Manuscript Proposal # 2960

PC Reviewed: 04/11/17  Status: _____  Priority: 2
SC Reviewed: _________  Status: _____  Priority: ____

1. a. Full Title: Adducin Mutations and Cognitive Impairments in Aging and Hypertension

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

2. Writing Group:
   Writing group members: Fan Fan
   Jeannette Simino
   Alexander P. Auchus
   Rebecca Gottesman
   David S. Knopman
   Thomas Mosley
   Michael Griswold
   Richard J. Roman

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

   First author: *Fan Fan, *Jeannette Simino
   * Equal contribution
   Address: 2500 North State Street
   Jackson, MS 39216

   Phone: 414-828-1008
   E-mail: ffan@umc.edu

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).
   Name: Thomas Mosley
   Address: 2500 North State Street
   Jackson, MS 39216

   Phone: 601 984 5610
   E-mail: tmosley@umc.edu
3. **Timeline:** Abstract to AHA High Blood Pressure Council 2017 Meeting: 6/1/2017 Meeting
   Manuscript: Dec 2017

4. **Rationale:**

Vascular cognitive impairment (VCI) is of growing relevance for the aging population in the United States. Evidence suggests that the myogenic response and autoregulation of cerebral blood flow (CBF) is often impaired in aging individuals, especially in patients with hypertension. This impairment may contribute to age-related cerebral vascular disease including VCI and Alzheimer’s disease. 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, blood brain barrier (BBB) leakage, and edema following elevations in CPP. However, very little is known about the genetic basis of impairments in CBF autoregulation and its role in cerebral vascular and neurodegenerative diseases.

In preliminary experiments, we identified a coding region sequence variant in the Add3 gene that impairs the myogenic response of cerebral arteries and autoregulation of cerebral blood flow in Fawn-hooded Hypertensive (FHH) rats. We created an Add3 transgenic knock-in FHH strain as well as Add3 KO rat strains and confirmed that loss of function mutations in Add3 modify cerebral blood flow hemodynamics. Moreover, we demonstrated that FHH rats with this mutation exhibit vascular remodeling, BBB leakage, cerebral inflammation, neurodegeneration, and learning and memory deficit as they age and develop hypertension. Mechanistically, Add3 forms a heterodimer with alpha-adducin (Add1) to regulate actin-spectrin interactions; this may alter potassium channel activity and vascular tone. Mutations in *ADD1* have been linked to hypertension and stroke in humans, but the role of adducin or *ADD3* in the regulation of cerebral vascular function and the development of mild cognitive impairment and dementia in aging and hypertensive patients has never been studied. In this proposal we will test for the association of *ADD1*, *ADD2*, and *ADD3* variants with hypertension, stroke, neurodegenerative disease as detected by MRI, and cognitive diagnoses (dementia, mild cognitive impairment (MCI), or normal cognitive function; vascular dementia/MCI). We will use whole exome sequence to perform single-variant and gene-based tests, as well as genome-wide association data to test the non-coding region variants previously associated with blood pressure.

5. **Main Hypothesis/Study Questions:**
Our overall goal is to determine if adducin genes (ADD1, ADD2, or ADD3) are associated with neurocognitive, cerebrovascular, or neuroimaging traits in ARIC participants. Our specific aims are as follows:

a. To identify common and rare ADD1, ADD2 or ADD3 variants associated with cognitive diagnoses (adjudicated cognitive status, cerebrovascular dementia/MCI), cerebrovascular traits (microbleed presence, infarct presence), or neuroimaging (temporal volume, AD signature region volume, and white matter intensity volume) phenotypes in ARIC African and European Americans.

b. To determine whether blood pressure, hypertension, lipid levels, plasma glucose concentration, the APOE ε4 genotype, race, or sex, modify the effect of adducin variants on neurocognitive, cerebrovascular, or neuroimaging traits.

We can conduct additional experiments in the FHH rat models to delineate the mechanisms underlying any observed genetic associations with neurocognitive, cerebrovascular or neuroimaging traits.

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:**
Cross-sectional candidate gene study of the ARIC fifth examination and ARIC-Neurocognitive participants

**Exclusion Criteria:**
- Participants missing outcomes, covariates, or genetic data
- 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 or Washington County, MD field centers
- European Americans (EAs) from the Jackson, Mississippi field center

**Outcomes (at visit 5 or NCS):**

Previously associated outcomes (Confirm these associations in our samples):
- Stroke
- Blood pressure (SBP, DBP, MAP, PP, Hypertension): log-transformed after medication adjustments when necessary
Hypothesized Cognitive Associations:
• Adjudicated cognitive diagnosis (dementia, mild cognitive impairment, and normal)
• Cerebrovascular cognitive impairment/dementia (dichotomized from etiology: any CVD involvement whether alone or in conjunction with AD, LBD, other)
• Infarct presence in MRI (Yes/No)
• Microbleed presence in MRI (Yes/No)
• Temporal volume
• AD signature region volume
• White matter hyperintensity volume (log2-transformed)

Covariates:

All outcomes will be adjusted for age (in years) at the fifth examination, sex, field center, and Eigenstrat-derived principal components (to control for population substructure). In addition, the minimal adjustments for specific traits will be as follows:

• Blood pressure traits will include age-squared and body-mass-index recorded at the fifth examination
• Adjudicated cognitive diagnosis and vascular dementia/MCI models will include education and the apolipoprotein E (APOE) ε4 genotype ascertained from TaqMan assays
• Temporal, AD signature region, 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 dummy variables indicating heterozygosity and homozygosity of the APOE ε4 allele.

Interaction models will include the main effects of the effect modifier (blood pressure, hypertension, lipid levels, plasma glucose concentration, APOE ε4 genotype). For all models, we will perform more extensive adjustments based on current standards in the literature. We fit the minimal models to check whether additional covariates mask the genetic effects (ex. adducin influencing the trait though blood pressure). For example, the maximally adjusted model for microbleeds may contain the adducin variant, age, sex, field center, principal components, education, hypertension status, ever smoking status, and diabetes.

Genetic Data:

We will extract ADD1, ADD2, and ADD2 variants from both the ARIC Freeze 5 whole exome sequence data (primarily contains protein-coding regions) and the genome-wide association (GWAS) data (1000 Genomes Imputed; contains variants in coding, non-coding, and proximal intergenic regions). Exome sequence data will take priority for variants available in both. Single-variant tests will be performed on polymorphisms with minor allele frequencies exceeding 0.01, while gene-based (SKAT and burden) tests will be performed only on variants with minor allele frequencies less than 0.05.
**Statistical Analysis:**
Under the premise that both hypertension and aging can inflict cerebrovascular changes, we will include all participants from the fifth visit. The interaction analysis of cognitive traits will examine the impact of blood pressure and hypertension status. For the analyses to confirm prior blood pressure associations, 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. If the medication-adjusted SBP and PP exhibit skewness and excessive kurtosis, we will apply a log-transform. Similarly, we will scrutinize the distributions of the brain volumes for outliers and normality, applying a log2-transformation to the white matter hyperintensity volume.

All analyses will be conducted separately by race. The 3-level cognitive diagnosis (dementia, MCI, normal) association will be evaluated using a multinomial logistic regression model. Dichotomous outcomes (stroke, hypertension, infarct presence, microbleed presence, cerebrovascular dementia/cognitive impairment) will be assessed through logistic regression, while blood pressures and brain volumes will be modeled via linear regression. We will assume additive effects for single-variant models of polymorphisms with minor allele frequencies exceeding 0.01, further performing SKAT and burden tests to collectively test rare variants. We will perform analyses both ignoring and accounting for the MRI selection scheme (for applicable phenotypes), genetic data availability, and attrition. Both the unweighted and weighted analyses will be programmed in Stata. However, ProbaABEL and seqMeta will be used to validate our results for the unweighted single-variant and gene-based tests, respectively. We will also correct for multiple testing using a conservative approach (Bonferroni).

**Limitations:**

- We have a small sample by genetic study standards, particularly to study interaction effects.
- Although pure vascular MCI/dementia is the ideal outcome, there are too few participants with this diagnosis to conduct a genetic analysis. Thus, we will 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? Yes
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_

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 # 717:** Adducin (ADD) Gly460Trp polymorphism and G-protein β3 subunit (GNB3)C825T polymorphism predict stroke case status (Lead author: Eric Boerwinkle)

**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)

Manuscript # 717: Adducin (ADD) Gly460Trp polymorphism and G-protein \( \beta \)3 subunit (GNB3)C825T polymorphism predict stroke case status (Lead author: Eric Boerwinkle)

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)

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. Yes, we understand and agree to this policy.

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 will abide by this 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.