1.a. Full Title: Identification of candidate genes associated with cardiovascular Disease (CVD) that predict cognitive change in mid-life: The Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): CVD genes and cognition

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

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3. **Timeline:**  
Statistical analyses: October 2010 –December 2010  
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4. **Rationale:**

The morbidity and mortality due to age-related neurological diseases including vascular and Alzheimer’s dementia (AD) are substantial and are predicted to increase as a result of the aging of the United States population. The results of studies in aging twins suggest that there is a significant genetic component underlying the variation between individuals in neuropsychological assessments of both verbal memory and executive function, cognitive domains that can show evidence of decline as much as ten years in advance of clinically diagnosed dementia.\cite{Elias, 2000} Higher concordance rates for these indices were found for monozygotic twins than for dizygotic twins with heritability or the percentage of similarity between twins that is attributable to genetic effects estimated to be as high as 68% or 56%, respectively.\cite{Carmelli, 2002, Finkel, 1995, McClearn, 1997, Swan, 1990, Swan, 1999, Swan, 2002, Plomin, 1994, Finkel, 1995, Benyamin, 2005}. Despite this evidence for a substantial genetic contribution to cognitive function, few genes other than APOE have been reproducibly associated with cognitive change in cohorts of community-dwelling adults.

Cognitive tests have been administered at four ARIC clinic visits between 1990-1992 and 2004-2006. The neuropsychological measures were chosen to represent three domains: verbal memory (Delayed Word Recall Test), executive function (Word Fluency Test), and psychomotor speed (Digit Symbol Substitution Test). The entire cohort has been genotyped for nearly 49,000 single nucleotide polymorphisms (SNPs) in 2,100 candidate genes as part of the shared Candidate Gene Association Resource (CARe) funded by the National Heart, Lung, and Blood Institute (NHLBI)\url{http://www.public.nhlbi.nih.gov/GeneticsGenomics/home/care/aspx/}. Genes and pathways implicated in cardiovascular disease as well as lipid metabolism, thrombogenesis, insulin resistance, metabolism, inflammation, oxidative stress, and apoptosis were of particular interest in the selection process for inclusion of genetic variants on a custom 50K genotyping array.\cite{Keating, 2008} The availability of these resources provides a timely opportunity for gene discovery when combined with assessment of interindividual differences in cognitive function and change.

Cardiovascular risk factors have been shown in large population-based studies including ARIC,\cite{Knopman, 2001} the Cardiovascular Health Study,\cite{Kuller, 1998} and the Rotterdam Study\cite{Breteler, 1994} to have an impact on cognitive function. ARIC was one of the first and largest studies to examine risk factors for cognitive status and decline in a middle aged population. In cross-sectional analyses, associations were found between cognitive status and age, sex, education, marital status,
depressive symptoms, diabetes, smoking, alcohol, fibrinogen level, pulmonary function, and carotid IMT. \cite{Cerhan, 1998} Six-year cognitive decline was independently associated with baseline hypertension, diabetes, interim strokes, and elevated insulin level. \cite{Alves de Moraes, 2002} A dose response relationship between \textit{APOE} genotype and cognitive decline was also found, with greater change as the number of \( \varepsilon4 \) alleles carried by an individual increased. ARIC was one of the first studies to show that the processes through which \textit{APOE} influences the risk for dementia may be operative years or decades before dementia becomes clinically apparent. \cite{Blair, 2005}

In 1993-1995 and 2004-2006, 1,945 and 1,025 study participants, respectively, underwent cerebral MRI and cognitive testing at the Forsyth County and Jackson field centers (R01 HL70825; T.H. Mosley PI). Brain images were graded both semi-quantitatively (at visit 3 and at Brain MRI follow-up visit) and quantitatively (follow-up visit only) for total brain, intracranial, white matter hyperintensity, ventricular, and hippocampal volumes. Testing included delayed word recall, digit-symbol substitution, and word fluency (visit 3 and Brain MRI follow-up visit) plus global mental status, subjective memory complaints, verbal and non-verbal memory, language, attention and processing speed, executive function, motor function, and neurological signs and symptoms (follow-up visit only). Expanding upon the previous analysis of 6-year change, multivariate random effects linear models revealed significant independent associations of five risk factors with 14-year cognitive decline, including \textit{APOE} genotype, diabetes, hypertension, incident stroke, and retinal vascular signs. \cite{Knopman, 2009, Lesage, 2009}

In the current study we propose to uncover genetic variation underlying both cross-sectional and longitudinal measures of cognitive function, and to evaluate whether vascular risk factors including type 2 diabetes and hypertension modulate the independent effect of SNPs or genes on the maintenance of cognitive function in mid-life. This may help to identify individuals who would especially benefit from risk factor modification and lead to more focused public health interventions.

References

5. **Main Hypothesis/Study Questions:**

The aims of the study are:

**Aim 1:** To identify the main effects of genes associated with cognitive function at visit 2 in middle-aged aged adults in the ARIC cohort who are free of known risk factors including clinical stroke that are known to impact cognitive function.

**Aim 2:** To identify the main effects of genes associated with cognitive change in participants who are free of known risk factors including clinical stroke that are known to impact cognitive function.
Aim 3: To examine whether any associations between cognitive function at visit 2 and genetic variants is modified in the context of cardiovascular risk factors by conducting tests of gene-environment interaction for both type 2 diabetes and hypertension status.

Aim 4: To examine whether the risk of cognitive decline conferred by genetic variants is modified in the context of cardiovascular risk factors by conducting tests of gene-environment interaction for both type 2 diabetes and hypertension status.

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).

Dependent variable/Cognitive variables:
The Delayed Word Recall Test (DWR), Digit Symbol Substitution Test (DSS), and Word Fluency Test (WF) are available from Visit 2 (1990-1992, labeled cognitive assessment 1 [CA1], whole cohort), Visit 3 (1993-1995, labeled CA2, Forsyth and Jackson MRI subset), Visit 4 (1996-1998, labeled CA3, whole cohort), and in participants in the ARIC Brain MRI study (2004-2006, labeled CA4, Forsyth and Jackson Brain MRI study subset).

For the subset (N = 1,134) of participants enrolled in the ancillary ARIC Brain MRI study a more extensive battery of neuropsychological tests was administered (2004-2006, CA4). From this battery, 5 domains of cognitive functioning were derived through principal components factor analysis. The factors to be examined in the current study are: (1) Global Mental Status, (2) Memory, (3) Psychomotor Speed, (4) Verbal Fluency, and (5) Executive Function.

Data Analytic Plan:
Screening of a panel of 2,100 candidate genes previously associated with cardiovascular and metabolic phenotypes will be performed separately in whites and African-Americans. Cognitive function at baseline (Aims 1 and 3) will be assessed using the results of neuropsychological tests administered at visit 2. Cognitive change (Aims 2 and 4) will be defined either as test score at visit 4 (1996-1999) – test score at visit 2 (1990-1992) (6-year change) or as test score at the ARIC Brain MRI follow-up visit (2004-2006) – test score at visit 2 (14-year change) for each of the three neuropsychological tests.

Multivariable linear regression and an additive genetic model for each SNP will be applied both for the cross-sectional analyses of cognitive function and to examine change in cognitive function. Primary analyses will adjust for age, gender, and educational level (basic model) and for age, gender, education, APOE genotype, diabetes, and hypertension (full model). In secondary analyses, multivariable logistic regression will model categorical measurement of cognitive status or change (e.g. below the 20th percentile of change scores for each of the 3 cognitive tests). An a priori significance threshold will be set at 2 x 10^{-5} after applying an expected false positive rate of 1 divided by the number
of tests performed (1/50,000). In a gene-based rather than SNP-based approach, p-values will be corrected for carrying out correlated tests for multiple SNPs across a single gene region using the $P_{ACT}$ method in secondary analyses.

For the analyses of interaction between candidate genes and cardiovascular risk factors (Aims 3 and 4), multivariable linear regression and an additive genetic model for each SNP will be applied to examine possible effect modification by diabetes/hypertension of the relationship between cognitive change considered as a quantitative trait and common genetic variants, with inclusion of interaction terms in the model. Primary analysis models will include 1) age, gender, education, genotype, diabetes status, and (diabetes x genotype) or 2) age, gender, education, genotype, hypertension, and (hypertension x genotype). In secondary analyses, multivariable logistic regression will model categorical measurement of cognitive change (e.g. below the 20th percentile of change scores for each of the 3 cognitive tests). Stratified analyses of the association between SNPs with evidence for interaction with the selected vascular risk factors will be performed in each of two groups of individuals classified on the basis of case status as a further investigation of effect modification.

All of the analyses described for Aims 1-4 will be performed by Jan Bressler under the supervision of Eric Boerwinkle; a signed data distribution agreement has been completed.

Inclusion/Exclusion:
We will exclude by DNA restriction, ethnic group (as appropriate to each field center), and missing data. Additional exclusion criteria will include missing data, neurologic, systemic, or psychiatric conditions that adversely influence cognition including a prior history of transient ischemic attack or stroke, depression, use of drugs affecting the central nervous system, and extremely low cognitive test scores at baseline that may be indicative of preclinical dementia.

Other variables of interest:
In Aims 1-4 above, we will determine whether any observed relationships are independent of cardiovascular risk factors and potential confounding factors. These factors will be taken primarily from the baseline examination (visit 2) and will include but are not limited to:

Visit 1: self-reported race, gender, exam center, education, prevalent stroke

Visit 2: age, lipid variables (total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides), BMI, waist-to-hip ratio, cigarette smoking, hypertension status, diabetes status, systolic and diastolic blood pressure, use of medication to control blood pressure and diabetes, APOE genotype, fasting blood sugar, fasting glucose, alcohol consumption, and insulin level

ARIC Brain MRI study: Depression as assessed as Vital Exhaustion at CA1 and the CES-D score
CNS medications (antidepressants, neuroleptics, antianxiolytics, benzodiazepines, antiepileptics) at each visit (visit 2, visit 3, visit 4, and ARIC Brain MRI Study)

Incident stroke

Study limitations:
A limitation of the study is the possibility of selection bias introduced because of differences between those subjects who did and did not participate in the Brain MRI study. To address this issue, baseline characteristics and clinical outcomes will be compared for the two groups.

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 ICTDER02 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 ICTDER02 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 ICTDER02 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.csecc.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)?

#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, Mayo Clinic, Rochester, MN)

#924 Apolipoprotein E genotype, cardiovascular risk factors, and cognitive decline in a middle-aged cohort: the Atherosclerosis Risk in Communities Study (Lead author: Cindy K. Blair, University of Minnesota, Minneapolis, MN)
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* AS#_1999.01)   
   ___ 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/

12. 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. Agree.