ARIC Manuscript Proposal # 1363

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SC Reviewed: __________   Status: _____   Priority: ____

1.a. Full Title: PCSK9 sequence variation and cognitive decline

b. Abbreviated Title (Length 26 characters): PCSK9 and cognitive decline

2. Writing Group:
   Writing group members: Jan Bressler, Thomas Mosley, Alan Penman, Dave Knopman, Eric Boerwinkle. Other investigators welcome.

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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4. Rationale:

   The proprotein convertase subtilisin/kexin type 9 serine protease gene (PCSK9) has been mapped to chromosome 1p32-1p34 and encodes a 692-amino acid glycoprotein
known as NARC1 (neural apoptosis-regulated convertase 1) \(^1\). \textit{PCSK9} is in the cholesterol secretory pathway and is most highly expressed in the liver, small intestine, and kidney \(^2\). Recently, three missence mutations in \textit{PCSK9} have been found to cause severe hypercholesterolemia \(^1,3,4\). Since overexpression of \textit{PCSK9} in the livers of mice results in a reduction in Ldr (low-density lipoprotein receptor) protein without changing the level of Ldr mRNA, these mutations probably confer a gain-of-function and lead to elevated cholesterol by reducing clearance of LDL (low density lipoprotein) from the circulation \(^5-7\). Cohen et al predicted that loss-of-function mutations would cause hypocholesterolemia and tested this hypothesis by sequencing the coding region of \textit{PCSK9} in subjects with low plasma LDL cholesterol (LDL-C) from the multi-ethnic Dallas Heart Study \(^8\). Two nonsense mutations (Y142X and C679X) were found at a combined frequency of approximately two percent among the African-Americans in the population and were associated with a 40% reduction in LDL-C. Mice deficient in \textit{Pcsk9} show a marked increase in Ldr protein and reduced plasma cholesterol so that inactivation of \textit{PCSK9} is thought to be equivalent to a loss-of-function mutation \(^9\). A missense mutation (R46L), associated with a 21% decrease in plasma LDL-C in white subjects in the same study, was described in a later report \(^10\).

The effect of a reduction in plasma LDL-C on the risk of coronary heart disease (CHD) was then examined in white and African-American participants in the large prospective Atherosclerosis in Communities Study (ARIC) by comparing CHD incidence over a 15-year interval in subjects with and without the relevant \textit{PCSK9} variants \(^11\). Incident CHD was defined as the occurrence of myocardial infarction, coronary death, or coronary revascularization. The Y142X and C679X nonsense mutations were found in 2.6% of African-American subjects and were associated with a 28% reduction in mean LDL-C and an 88% reduction in the risk of CHD; similarly, there was a 15% reduction in LDL-C and a 47% reduction in the risk of CHD in the 3.2% of Caucasian subjects who had the \textit{PCSK9} R46L polymorphism. The observed reductions in the risk of CHD exceeded those shown in clinical trials of statins and suggest that early intervention with lipid lowering agents may be beneficial.

Cardiovascular risk factors have been shown in large population-based studies including ARIC \(^12\), the Cardiovascular Health Study \(^13\), and the Rotterdam Study \(^14\) to have an impact on cognitive function. The aim of this proposal is to evaluate whether decline in cognitive function will be reduced for Caucasian and African-American participants inheriting \textit{PCSK9} genetic variants associated with lower LDL-C levels when compared to subjects carrying the major allele at each locus. Although hyperlipidemia measured at the baseline cognitive assessment (Visit 2) in ARIC was not previously found to be associated with change in cognitive test scores over a six-year period (Visit 2 – Visit 4) \(^15\) the absolute amount of decline measured in this group of middle-aged adults was small. The availability of cognitive data from a 14-year follow-up period for 1134 subjects participating in the ARIC Brain MRI study (2004-2006) warrants further investigation.

The hypothesized relationship between cognitive function and lifelong reduction in LDL-C levels is supported by some but not all studies of the association between plasma lipid levels and change in cognition \(^16\). For example, increasing cognitive performance was found for women with cardiovascular disease with decreasing quartiles of LDL cholesterol \(^17\). Similarly, in a random sample of 111 subjects aged 65-84 years from the
general population enrolled in the Rotterdam Study, plasma cholesterol levels were
significantly associated with the presence of white matter lesions that were predictive of
lower scores on tests of cognitive function. In contrast, in a prospective study of 1147
individuals in the multiethnic Washington Heights-Inwood Cognitive Aging Project there
was no observed association between LDL-C and decline in memory, visuospatial ability,
or language.

An alternative perspective is provided by the authors of the report examining the
relationship between PCSK9 variants, reduction in LDL-C, and CHD. Cohen et al.
suggested that while an effect on cardiovascular risk is not captured in a single
measurement of plasma LDL cholesterol at baseline the association between the PCSK9
variants and decreased incidence of CHD can be explained as the influence of the
nonsense and missense PCSK9 mutations on cholesterol levels over the course of a
lifetime. A similar argument can be made here that an association of cognitive function
with PCSK9 polymorphisms may be revealed even if there is no demonstrated
relationship with plasma lipid variables for the longer follow-up period. The significance
of the proposed study lies in the fact that plasma cholesterol levels are modifiable by the
use of cholesterol-lowering medications and changes in diet, that changes in cognition
may be more marked as the middle-aged ARIC population progresses into older age, and
in the public health importance of increasing understanding of the factors affecting
cognitive function in the rapidly aging U.S. population.

References
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8. Cohen, J. et al. Low LDL cholesterol in individuals of African descent resulting from frequent
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10. Kotowski, I. K. et al. A spectrum of PCSK9 alleles contributes to plasma levels of low-density

5. Main Hypothesis/Study Questions:

The aims of the study are:

Aim 1: To determine if mutations in the \textit{PCSK9} gene are associated with cognitive status. Cognitive status will be defined by cognitive test scores (DWR, DSS, and WF) at Visit 2, Visit 4, and scores in 5 (factor-derived) cognitive domains assessed at the ARIC Brain MRI Visit (2004-2006).

Aim 2: To determine if mutations in the \textit{PCSK9} gene predict change in cognitive function over a 6 or 14-year follow-up period for the DWR, DSS, and WF tests.

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).

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 = 1134) 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.
Independent Variable:

Three sequence variants in PCSK9 (Y142X, C679X, and R46L) have been genotyped in the entire ARIC cohort. In a preliminary data analysis the race-specific prevalence of each mutation in the main cohort was too low for separate analysis by mutation (see table):

<table>
<thead>
<tr>
<th></th>
<th>Whites</th>
<th>Blacks</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCSK9R46L</td>
<td>281</td>
<td>18</td>
<td>299</td>
</tr>
<tr>
<td>PCSK9Y142X</td>
<td>1</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>PCSK9C679X</td>
<td>5</td>
<td>34</td>
<td>39</td>
</tr>
<tr>
<td>Total</td>
<td>287</td>
<td>72</td>
<td>359</td>
</tr>
</tbody>
</table>

Therefore, in all analyses mutations will be pooled. Carriers of the minor allele of the Y142X and C679X polymorphisms will be pooled for statistical analysis in African-Americans (presence of any PCSK9Y142X or C679X mutation vs. absence). Heterozygous and homozygous carriers of the R46L polymorphism will be pooled for statistical analysis in Caucasians (presence of any PCSK9R46L mutation vs. absence).

Data Analytic Plan:

Caucasian and African-Americans will be evaluated separately. Linear regression will be used to test whether PCSK9 genotypes are associated with cognitive status on individual test scores at CA1 and CA3, and with domains scores at CA4.

Linear regression models will be applied to examine mean change in cognitive function between CA1 and CA3 (6 year change) for the entire ARIC cohort. Cognitive change will be defined as CA3 test score minus CA1 test score for each of three cognitive tests. Likewise, we will examine mean change in cognitive function between CA1 and CA4 (14 year change) for participants in the ARIC Brain MRI Study.

Multivariable logistic regression will be used to predict the association of sequence variation in PCSK9 with a categorical measurement of cognitive impairment, defined as those falling below the 10th percentile of scores for each of the cognitive domains at CA4.

Linear mixed models will also be fit to estimate the effect of PCSK9 mutations on the rate of change in cognitive function (slope) for the three cognitive tests performed at CA1, CA2, CA3, and CA4. This model assumes that an individual’s initial level of cognitive performance (intercept) and rate of change (slope) over time follow those of the population with the exception of random effects that contribute to variability in the intercept and slope. The use of the mixed model accounts for the correlation between cognitive test scores at repeated assessments while also allowing a more precise estimate of the error of variability.

The basic model will consist of terms for the PCSK9 genetic variants, age (years centered at 65 years), sex, education, time in years since baseline, and the interaction of time with PCSK9 genotype. The term for time refers to the annual rate of change in cognitive test score in the reference group (PCSK9 variant) and the interaction term reflects the additional effect of the PCSK9 polymorphism on the annual rate of change.

Inclusion/Exclusion:
We will exclude by DNA restriction, ethnic group (as appropriate to each field center), and missing data. Other exclusion criteria will include history of stroke or TIA prior to Visit 2, incident stroke, and use of lipid-lowering drugs. We will exclude those individuals with the lowest 5% of scores on the cognitive tests at CA1 to exclude those with possible preclinical dementia.

Other variables of interest:
In both aims 1 and 2 above, we will determine whether any observed relationships are independent of cardiovascular risk factors and potential confounding factors. These factors include:

Visit 1- Education, gender, exam center, systolic BP, diastolic BP, fasting glucose.

Visit 2- Age, occupational status, APOE genotype, fasting blood sugar, history of diabetes, fasting glucose, smoking pack years, hypertension status, antihypertensive medications, systolic blood pressure and diastolic pressure, BMI, carotid IMT(right and left sides), alcohol consumption, total cholesterol, LDL-c, HDL-c, triglycerides, Lp(a).

Depression as assessed as Vital Exhaustion at CA1 and the CES-D score at CA4. CNS medications (antidepressants, neuroleptics, antianxiolytics, benzodiazepines, antiepileptics) at each visit (CA1, CA2, CA3, CA4).

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.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)?

#314 Cerebral abnormalities identified on magnetic resonance imaging and cognitive functioning: the ARIC study (Lead author: Thomas Mosley, University of Mississippi, Jackson, MS)

#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)

#1093 PCSK9 mutations lower CHD risk (Lead author: Jonathan Cohen, University of Texas Southwestern Medical Center, Dallas, TX)

#1121 Cognitive change over 12 years and its relationship to cardiovascular risk factors: ARIC-MRI Study (Lead author: David Knopman, Mayo Clinic, Rochester, MN)

#1210 PSCK9 variants and cancer (Lead author: Aaron Folsom, University of Minnesota, Minneapolis, MN)

#1275 PCSK9 variants and PAD (Lead author: Aaron Folsom, 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.cscc.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.