ARIC Manuscript Proposal # 1321

PC Reviewed: _12/_11_/07	**Status: __A__**	**Priority: _2__**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Association of a SERPINA9 gene variant with carotid artery atherosclerosis: The Atherosclerosis Risk in Communities (ARIC) Carotid MRI Study

b. Abbreviated Title (Length 26 characters): SERPINA9 gene with carotid MRI

2. Writing Group:

Writing group members: Weihong Tang, Alanna Morrison, Bruce Wasserman, Aaron Folsom, CC representative, Eric Boerwinkle …

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

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3. Timeline:

Starting Analyses: December 15, 2007
First Draft: March 15, 2008
Submission for Publication: May 15, 2008

4. Rationale:

Vulnerable atherosclerotic plaques are plaques that have a high risk to cause cardiovascular complications such as myocardial infarction or stroke[1]. Recently published consensus documents included 5 major criteria to define vulnerable plaques based on the study of "culprit" plaques, including active inflammation, thin cap with large lipid-necrotic core, endothelial denudation with superficial platelet aggregation, fissured plaque, and stenosis larger than 90%[2,3]. Plaques with these characteristics have a higher risk to rupture compared to "stable" plaques. Therefore, it is important to identify factors contributing to the formation of vulnerable plaques. The use of magnetic resonance imaging (MRI) in the research of atherosclerosis allows direct visualization of diseased vessel wall and characterization of plaque composition and morphology with good accuracy and reproducibility[1]. This information is crucial in identifying vulnerable plaques, as demonstrated in a recent prospectively study in which the following plaque characteristics identified by MRI predicted symptoms of cerebrovascular events: presence of a thin or ruptured fibrous cap, intraplaque hemorrhage, larger mean intraplaque hemorrhage area, larger maximum %lipid-rich/necrotic core, and larger maximum wall thickness[4].

Evidence from animal and human studies suggest that inflammation is not only important for the development of atherosclerotic plaques but also their destabilization[5]. Studies of transgenic mice given splenectomy or bone marrow transplantation showed that during atherogenesis autoimmunity plays both detrimental and protective roles, with the former attributed to the involvement of T cells and macrophages and the latter to B cells[6,7]. The inflammation marker CRP is related to the extent of plaques measured by MRI in both thoracic and abdominal aortas[8] and also related to the mean numbers of thin cap atheromas in an autopsy study of sudden cardiac death[9].

In the ARIC study, the SNP rs11628722 in SERPINA9 gene, which results in amino acid change from Val to Ala, was significantly associated with incident ischemic stroke in both Blacks and Whites (Morrison et al, MS #1201). The hazard rate ratio of stroke associated with at-risk allele was 1.32 in Whites and 1.27 in Blacks after adjusting for traditional risk factors ($p<0.05$). The SERPINA9 gene encodes a protein named SERPINA9, also known as centerin or GCET1 [germinal center (GC) B-cell-expressed transcript 1][10,11]. SERPINA9 is a member of a large serpins (serine protease inhibitors) family consisting of at least 16 clade subfamilies with divergent functions[12]. SERPINA9 belongs to the clade A subfamily and is located on chromosome 14q32[13]. Two other members of the clade A, SERPINA8 (angiotensinogen) and SERPINA10 (protein Z-dependent protease inhibitor), have been associated with hypertension and venous thromboembolic disease, respectively[11]. Several other members of serpins, including plasminogen activator inhibitor-1 (PAI-1) of clade E and antithrombin of clade C, are involved in the regulation of thrombosis and fibrinolysis[14]. Northern blot analysis of human tissues showed that SERPINA9 was only detected in human lymph node [10] and spleen tissues (www.genecards.org). In lymph node sample, transcription of centerin was highly expressed in GC B-cells and was up-regulated by CD40 signaling, which is fundamental for GC formation[10]. Therefore, it was speculated that centerin plays an important role in maturation and maintenance of naïve B cells[10]. While mechanisms mediating the association between the SERPINA9 gene variant and stroke are unknown, it is possible that the association is mediated via the B cell pathway since B cells are

involved in atherogenesis. In addition, a recent study of transgenic mice reported that SERPINA9 was significantly up-regulated in the hippocampus tissues from Alzheimer's disease mice[15], suggesting the possibility of a direct involvement of SERPINA9 in the ischemic reaction of brain tissue.

The aim of this project is to test the hypothesis that the SERPINA9 rs11628722 variant is associated with carotid artery atherosclerotic plaque characteristics measured by MRI in a biracial cohort of adults from the ARIC Carotid MRI study.

5. Main Hypothesis/Study Questions:

The SERPINA9 rs11628722 variant is associated with features of carotid artery atherosclerotic plaques measured by MRI, with priori hypotheses related to maximum wall thickness, lipid core measures, and fibrous cap measures.

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

Outcome: MRI variables for maximum wall thickness, lipid core presence and size measures, and fibrous cap measures: GDSICA_TOTALWALLVOLUME1, GDSICA_TOTALWALLVOLUME1ADJ, GDSICA_MAXWALLTHICK_MAXCORE1, GDSICA_MAXWALLTHICK_SEG, MEAN_CAP_THICKNESS_2ADJACENT, MEAN_MIN_CAP_THICKNESS_2ADJACENT, GDSICA_TOTALLIPIDCOREVOLUME, GDSICA_MAXLIPIDCOREAREA, GDSICA_TOTALLIPIDCOREVOLUME_NEW2, GDSICA_MAXLIPIDCOREAREA_NEW2, and LIPID_CORE

Exposure: the SERPINA9 rs11628722 variant

Covariates include, but are not limited to, traditional risk factors including age, sex, race, total cholesterol, LDL, BMI, smoking status, diabetes, and hypertension.

Analysis Plan

Data analysis to be conducted by the coordinating center using SUDAAN.

1) Test of genotypes in Hardy-Weinberg equilibrium will be conducted on race-specific datasets using a modified chi-square test that takes into account of the sample weighting.
2) The primary SNP model will be an additive effect model in which genotypes will be coded as 0, 1, or 2 copies of the at-risk allele. If appropriate, a dominant model will be also tested.
3) Linear and logistic regressions will be carried out in SUDAAN and the analyses will be weighted by the inverse of the sampling fractions in the 8 sampling strata to test the null hypothesis that the phenotypic levels are not associated with genotypes.
4) The analyses in 3) will be conducted in Blacks and Whites separately. Interactions between race and gene on MRI phenotypes will be tested. When there are no significant race interactions, the analyses will be repeated on race-combined datasets.

5) In a secondary analysis, participants with a history of prevalent stroke, CHD, or a transient ischemic attack will be excluded, and the analyses in 3) and 4) will be repeated after the exclusion.

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 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 ____ 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)?

1. Manuscript #1201: Single nucleotide polymorphisms associated with coronary heart disease predict incident ischemic stroke in the Atherosclerosis Risk in Communities (ARIC) study
2. Manuscript #1204: MMP2 genetic variation influences measures of fibrous cap thickness: The Atherosclerosis Risk in Communities Carotid MRI Study

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*: ARIC carotid MRI study)

___ 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.

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