ARIC Manuscript Proposal #1941

PC Reviewed: 5/8/12  Status: A  Priority: 2
SC Reviewed: ________  Status: _____  Priority: ____

1.a. **Full Title:** Relationship between circulating levels of SDF alpha and carotid plaque characteristics: the Atherosclerosis Risk in Communities (ARIC) Carotid MRI Study

b. **Abbreviated Title (Length 26 characters):** SDF alfa and carotid plaque

2. **Writing Group:**
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I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. RTD

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**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).

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3. **Timeline:** Analysis will start as soon as the manuscript proposal has been approved. We anticipate journal submission of the completed manuscript within 1 year after manuscript proposal approval.

4. **Rationale:**

Stromal cell-derived factor-1 (SDF-1), also known as Chemokine (CXC motif) ligand 12 (CXCL12), is a chemokine involved in hematopoiesis, organ development, and angiogenesis. SDF alpha is also highly expressed in atherosclerotic plaques (1). Recently Genome-wide association studies (GWAS) have found highly significant associations between variants at the CXCL12 (10q11) locus and risk of myocardial infarction and coronary heart disease (CHD) (2). These variants are significantly associated with reduced plasma levels of CXCL12 (3). In a recent study, low plasma levels of SDF alpha were seen in patients with unstable angina (4). The goal of this study is to assess the relationship of plasma SDF alpha levels with atherosclerotic plaque burden and high risk plaques measured by carotid MRI. We will also assess the association of SDF alpha levels with high sensitivity C reactive protein (hs-CRP). We will perform a GWAS to identify the locus that controls the plasma levels of SDF alpha. Next we will assess if single nucleotide polymorphisms (SNP) found with the GWAS are associated with the atherosclerotic plaque burden and the high risk plaques.

**Background:**

SDF-1, a CXC chemokine, was originally identified as a bone marrow SDF from stromal cells which includes immune cells, pericytes, endothelial cells, inflammatory cells and fibroblasts. SDF-1 has been found to be a potent chemo-attractant for lymphocytes and monocytes in vitro and subsequently in vivo, and functions by binding to its sole receptor, CXCR4 (5). SDF-1 alpha is highly expressed in human atherosclerotic plaques and effectively activates platelets in vitro (1). Recent evidence implies that bone marrow derived cells contribute to neointima formation by giving rise to neointimal smooth muscle cells after arterial injury (6). The smooth muscle cell progenitors and SDF - alpha are both important contributors to neointima formation after arterial injury (7). After arterial injury smooth muscle cells express SDF- alpha which has been found to be involved in recruitment of peripheral blood progenitor cells which may contribute to neointima formation (8). Beside recruitment of peripheral progenitor cells, SDF alpha has also a role in the “memory” T cell arrest on the smooth muscle cells (9). Recently, it has been shown that blockade of the CXCR4 receptor results in accelerated atherosclerosis in mice (10). Because SDF alpha is thought to contribute to arterial repair and its receptor blockade results in accelerated atherosclerosis it is believed that SDF alpha may have a protective role against atherosclerosis by promoting survival mechanisms that counteract apoptosis and facilitate endothelial and vascular integrity (7, 10).

One study demonstrated significantly altered SDF-alpha and CXCR4 expression in patients with angina, with low SDF-alpha levels in plasma and altered expression of its corresponding receptor (4). Recent GWAS have identified variants at the 10q11 locus in
association with CHD risk. These variants are located within ~80kb upstream of chemokine (C-X-C motif) ligand 12 (CXCL12) gene, which encodes for SDF-1, now known more commonly as CXCL123. The relationship of SDF alpha with atherosclerotic plaque measurements has not been explored before.

The primary aim of this study is to assess the relationship of plasma SDF alpha levels with total atherosclerotic plaque burden (carotid wall thickness, wall volumes and normalized wall index) as assessed by carotid MRI. A secondary goal is to assess the relationship between SDF alpha levels and markers of high-risk plaques measured by carotid MRI (presence of lipid rich core, fibrous cap thickness). We hypothesized that low plasma SDF alpha levels would be associated with an increased carotid plaque burden and high-risk plaques. We will also assess the relationship between plasma SDF alpha levels and high-sensitivity C-reactive protein (hs-CRP). A third goal is to identify the locus that controls plasma SDF levels and to assess if the SNPs found, are associated with increased carotid plaque burden and high-risk plaques.

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

**Hypotheses:**

1. SDF alpha measured in the ARIC carotid MRI study visit will be negatively associated with increased atherosclerotic plaque burden measured by carotid MRI (carotid wall thickness, wall volumes and normalized wall index).
2. Plasma levels of SDF alpha will be negatively correlated with carotid MRI markers of high risk plaque (presence of lipid rich core, fibrous cap thickness).
3. SDF alpha levels will be correlated with the hs-CRP levels.
4. The association between SDF alpha and carotid arterial plaque burden (or high risk plaques) will be maintained after adjustment for traditional risk factors and hs-CRP.
5. Genetic variants associated with low plasma SDF alpha levels are associated with increased atherosclerotic plaque burden and high risk plaques.

**Study questions:**

1. Are SDF alpha levels negatively associated with the carotid plaque measurements assessed with carotid MRI?
2. Are SDF alpha levels negatively associated with the carotid MRI markers of high risk plaque?
3. Are SDF alpha levels associated with plasma levels of hs-CRP?
4. Is the negative association between SDF alpha and measures of plaque burden (and plaque vulnerability) independent of the traditional risk factors and hs-CRP?
5. What are the genetic loci that control plasma SDF alpha levels?
6. Are the genetic variants that control plasma SDF alpha levels associated with plaque burden and high risk plaques?

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).

Overview: To test our hypotheses, we will utilize the carotid plaque MRI measurements performed at the ARIC Carotid MRI visit and the plasma samples collected at the same visit.

Plasma levels of SDF alpha have been measured in the entire ARIC Carotid MRI cohort. Of the 2066 ARIC cohort members who participated in the Carotid MRI substudy, 1901 had a complete MRI exam, of which 1769 had sufficient quality of MRI scans and adherence to MRI protocol to be included for analyses.

We are interested in the following variables in the ARIC database measured at ARIC Carotid MRI visit:

**Independent variables**: age, gender, race, body mass index, smoking status, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, hs-CRP, creatinine, estimated glomerular filtration rate, systolic blood pressure, presence of diabetes (fasting blood sugar ≥ 126 mg/dl or use of diabetes medication), use of antihypertensive medications, use of diabetes medications, use of aspirin, use of cholesterol lowering medications, SDF alpha levels,

**Dependent variables:**

**Measures of plaque burden**: Carotid wall thickness, Total wall volume (GDISCA-TOTAL WALL VOLUME), Maximal wall thickness (GDSICA-MAXWALLTHICK-MAXCORE), Lumen area (LUMENAREA_MAXMEANWALL1), Vessel wall area (VESSELWALL AREA_MAXMEANWALL1), Normalized wall index (NWI) = wall area/ total vessel wall area (as an index of positive remodeling response)

**Measures of plaque composition**:

Lipid core: Total lipid core volume (GDSICA-TOTALLIPIDCOREVOLUME), Max lipid core area (GDSICA-MAXLIPIDCOREAREA-NEW2), lipid core (present/absent) (LIPID_core), Lipid core present in two adjacent slices (CORE_in_two) (note: restricted to those with maximum wall thickness ≥ 1.5 mm)

Fibrous cap thickness: Mean cap thickness (MEAN-CAP-THICKNESS-2ADJACENT) Mean minimum cap thickness (MEAN-MIN-CAP-THICKNESS-2ADJACENT) (note: restricted to participants with lipid core present)

For analysis of the association between SDF alpha levels and the continuous carotid MRI measurements as well as with hs-CRP we will use linear regression models. We will model SDF alpha as both categorical and a continuous variable (absolute levels or log
levels if the distribution is skewed). For analyses based on categorical SDF alpha levels, we will divide up SDF alpha levels by quintiles or quartiles and compare the highest with the lowest quintile (or quartile). Quartile 1 will be used as the referent quartile. Standardized regression coefficients (beta-coefficients) will be presented for linear regression models, which will be standardized by 1 SD of exposure and outcome with adjustment for covariates. For categorical outcome (like presence or absence of lipid rich core), we will perform logistic regression analyses. For the association of the plasma levels of the SDF alpha and hs-CRP we will use linear regression models to calculate the correlation coefficients.

Genome-wide genotyping of single-nucleotide polymorphisms (SNPs) will be performed using the Affymetrix Genome-Wide Human SNP Array 6.0 (Santa Clara, California). To test the association of genetic variants with the outcomes we will use linear regression for the continuous variable and logistic regression for the binomial outcomes.

To account for covariates, we will create 3 adjustment models:

- Model 1 will be a basic model adjusted for age, gender, and race.

- Model 2 will be adjusted for: all variables in Model 1 plus LDL-C, high density lipoprotein cholesterol, systolic blood pressure, antihypertensive medication use, smoking status and the presence of diabetes mellitus (fasting blood glucose > 126 mg/dl or diabetes medication use), BMI, aspirin, history of cardiovascular disease (coronary heart disease and ischemic stroke).

- Model 3 will be adjusted for all the factors included in model 2 plus hs-CRP, and use of cholesterol lowering medications.

Depending on the strength of the associations in the primary analyses, we will perform stratified analysis by other variables such as: age or use of statins

**Inclusion criteria:**
Patients who had sufficient quality of MRI scans and adherence to MRI protocol to be included for analyses at the ARIC carotid MRI study visit.

**Exclusion criteria**
Patients who met the ineligibility criteria for the Carotid MRI substudy: standard contraindications to the MRI exam or to the contrast agent, carotid revascularization on either side for the low CIMT group or on the side selected for imaging for the high CIMT group, and difficulties in understanding questions or in completing the informed consent.

**Limitations:**
Some of the limitations of this study include the fact that this is an observational study. The other limitation is that the SDF alpha levels and the carotid MRI were measured at
the same time. Because of that we will not be able to assess the relationship between the timing of low levels of SDF alpha and the plaque characteristic.

7.a. Will the data be used for non-CVD analysis in this manuscript?  _____ 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?  _____ Yes  
        _____ No
        (This file ICTDER03 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  
        _____ 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”?  _____ 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.cscce.unc.edu/ARIC/search.php  
        _____ 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)?

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?  _____ Yes  
        _____ No

11.b. If yes, is the proposal  
        _____ A. primarily the result of an ancillary 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.cscce.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.

References: