ARIC Manuscript Proposal #2062

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SC Reviewed: _________  Status: _____  Priority: ____

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

b. Abbreviated Title (Length 26 characters): Osteopontin 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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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:**

Osteopontin (OPN) is a non-collagenous matricellular protein that has an important role in cardiac remodelling. Recent studies have shown that OPN plays an important role in inflammation and tissue remodelling via its action as an inflammatory cytokine. Other studies have shown that OPN mRNA expression in human atherosclerotic plaques, are closely associated with the severity of atherosclerosis and calcification. Plasma OPN levels have been also found to be associated with the presence and extent of coronary artery disease. Plasma OPN levels were also shown to be significantly positively correlated with atherogenic lipid profile, hsCRP, mitral annular calcification grading, aortic valve sclerosis grading, and the number of stenosed coronary vessels in coronary artery disease (CAD) patients. Preprocedural OPN levels in patients undergoing PCI have shown to be independent predictors of post procedure cardiovascular events. The goal of this study is to assess the relationship of plasma OPN levels with atherosclerotic plaque burden and calcification and high-risk plaques measured by carotid magnetic resonance imaging (MRI). We will also assess the association of OPN levels with high sensitivity C reactive protein (hs-CRP). We will perform a genome wide association study (GWAS) to identify the locus that controls the plasma levels of OPN. Next we will assess if single nucleotide polymorphisms (SNP) found with the GWAS are associated with the atherosclerotic plaque burden, calcification and the high-risk plaques.

**Background:**

OPN is a non-collagenous matricellular protein that has an important role in cardiac remodelling. It is an aspartic acid-rich, N-linked glycosylated protein that may be highly phosphorylated on serines and threonines depending on the cell type. In the adult human, OPN protein is generally restricted to bones, kidneys and the epithelial linings. In the atherosclerotic lesions, OPN is expressed in smooth muscle cells in the lesion, in angiogenic endothelial cells, and in macrophages. Consistently, animal models have confirmed the role of OPN in vascular remodeling by showing that OPN was expressed in intimal smooth muscle cells undergoing proliferation and migration. Anti-OPN antibodies also inhibited smooth muscle cells accumulation in intima, and forced overexpression of OPN resulted in thickening of the medial layer and increased neointima formation following injury. All these data indicate that during injury, OPN modulates the proliferation, migration, and accumulation of smooth muscle and endothelial cells involved in repair and remodeling processes of the vasculature. Other studies in mice that have overexpression of OPN show enhancement of atherosclerotic lesion. On the other hand mice that have loss of function of OPN gene have
significantly smaller atherosclerotic lesions \(^{17,18}\). Together, these data support the notion that OPN is an important participant in atherosclerotic plaque formation.

In clinical studies on humans plasma OPN levels have been found to be associated with coronary artery disease \(^7\). Ohmori et. al. found that patients with CAD have higher levels of OPN than patients without CAD (616±308 ng/ml versus 443±237 ng/ml, \(P<0.001\)). He also found a stepwise increase in OPN levels depending on the number of >50% stenotic coronary vessels: 540±293 ng/ml in 1-vessel, 615±230 ng/ml in 2-vessel, and 758±416 ng/ml in 3-vessel disease. In this study in multivariate analysis, OPN levels were significantly associated with CAD (odds ratio=1.21, 95% Confidence Interval (CI) =1.05–1.39 for a 100 ng/ml increase) independent of traditional risk factors. OPN levels were higher in patients with calcification than in those without calcification (608±328 ng/ml versus 490±246 ng/ml, \(P<0.01\)) and correlated with the number of calcified segment (r=0.26, \(P<0.001\)) \(^7\). In another study by Kato et. al. on patients with CAD who had angiography performed, OPN levels at the time of the angiography were higher in patients with major adverse cardiac events (MACE) 3 years after the procedure as opposed to those without events (586±230 vs 438±195 ng/ml, \(p<0.005\)). OPN was also an independent predictor of MACE (Hazard Ratio 1.3, 95% CI 1.1–1.5, for a 100 ng/ml increase in OPN) \(^9\). Studies on human aortic aneurysms revealed that the OPN gene expression was increased in smooth muscle cells of the thoracic aortic aneurysms \(^{19,20}\). One study by Lorenzen et al. showed that OPN is negatively correlated with Estimated Glomerular Filtration Rate (eGFR), suggesting that assessment of renal function is very important in interpreting OPN in patients with cardiovascular disease \(^21\). Other studies have shown that OPN levels are decreased by statin use, angiotensin receptor blocker use and spironolactone use,\(^ {22,23}\).

The primary aim of this study is to assess the relationship of plasma OPN levels with total atherosclerotic plaque burden (carotid wall thickness, wall volumes and normalized wall index) and calcification (maximum calcified volume and area of calcification) as assessed by carotid MRI. A secondary goal is to assess the relationship between OPN levels and markers of high-risk plaques measured by carotid MRI (presence of lipid rich core, fibrous cap thickness). We hypothesized that high plasma OPN levels would be associated with an increased carotid plaque burden, calcification and high-risk plaques. We will also assess the relationship between plasma OPN levels and high-sensitivity C-reactive protein (hs-CRP). A third goal is to identify the locus that controls plasma OPN 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. Plasma levels of OPN measured in the ARIC carotid MRI study will be positively associated with increased atherosclerotic plaque burden measured by carotid MRI (carotid wall thickness, wall volumes and normalized wall index).
2. Plasma levels of OPN will be positively correlated with the maximum calcium volume and with the maximum calcification area.

3. Plasma levels of OPN will be correlated with carotid MRI markers of high-risk plaque (positive correlation with presence of lipid rich core and negative correlation with fibrous cap thickness).

4. Plasma levels of OPN levels will be positively correlated with the hs-CRP levels.

5. The association between plasma levels OPN and carotid arterial plaque burden (or high risk plaques) will be maintained after adjustment for traditional risk factors and hs-CRP.

6. Genetic variants associated with plasma OPN levels are associated with increased atherosclerotic plaque burden and high-risk plaques as assessed by carotid MRI.

Study questions:

1. Are OPN levels positively associated with measures of carotid plaque burden assessed with carotid MRI?

2. Are OPN levels positively associated with plaque calcifications assessed with carotid MRI?

3. Are OPN levels positively associated with the carotid MRI markers of high-risk plaque?

4. Are OPN levels associated with plasma levels of hs-CRP?

5. Is the association between OPN levels and measures of plaque burden (and plaque vulnerability) independent of the traditional risk factors and hs-CRP?

6. What are the genetic loci that control plasma OPN levels?

7. Are the genetic variants that control plasma OPN levels also associated with plaque burden, calcification burden and high-risk plaques, as assessed by carotid MRI?

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 OPN have been measured in the entire ARIC Carotid MRI cohort. Of the 2066 ARIC cohort members who participated in the Carotid MRI sub study, 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 eGFR, 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, stromal cell-derived factor (SDF) alpha levels, osteopontin levels, calcium 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 calcification:** Maximum calcification volume, Area of calcification

**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 evaluating the association between OPN levels and the continuous carotid MRI variables as well as with hs-CRP we will use linear regression models. We will model OPN as both categorical and a continuous variable (absolute levels or log levels if the distribution is skewed). For analyses based on categorical OPN levels, we will divide up OPN 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 OPN 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 MRI variables we will use linear regression for the continuous variable and logistic regression for the binominal outcomes. Because of the small number of African American participants we will perform GWAS analysis only in Caucasians.

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) and eGFR.

- **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, use of statin, use of angiotensin receptor blockers or use of spironolactone.

**Inclusion criteria:**
Patients who had sufficient quality of MRI scan 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 sub study: 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 OPN 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 levels of OPN and the plaque characteristic.

**7.a. Will the data be used for non-CVD analysis in this manuscript?**

- X 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?    __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 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.cscn.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)?

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?    ____ Yes    __X_ 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.cscn.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:


22. Lorenzen JM, Neunhoffer H, David S, Kielstein JT, Haller H, Fliser D. Angiotensin ii receptor blocker and statins lower elevated levels of osteopontin in essential hypertension--results from the eutopia trial. Atherosclerosis. 2010;209:184-188