ARIC Manuscript Proposal # 1329

PC Reviewed: 01/15/08	**Status: __A__**	**Priority: __1_**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Relationship Between Circulating Levels of Matrix Metalloproteinases (MMPs) and Carotid Artery Plaque Characteristics: The ARIC Carotid MRI Study

b. Abbreviated Title (Length 26 characters): MMPs and Carotid Artery Plaque

2. Writing Group:

Writing group members: John W. Gaubatz, Ron C. Hoogeveen, Woody Chambless, Bruce Wasserman, Christie M. Ballantyne, Eric Boerwinkle. Others are welcome.

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

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3. Timeline: Data analyses will start immediately following approval of the manuscript proposal. Completion of a first draft of the manuscript is anticipated within 3 months of completion of all data analyses. A final version of the manuscript will be submitted for publication to a peer reviewed journal within 4 months of the data analyses start date.

4. Rationale:

Atherosclerosis is a complex inflammatory process that is characterized by the formation of raised lesions resulting in the narrowing of the lumen in a number of arterial beds (Ross, 1999). In the initial stages of lesion formation the plaque may consist solely of a fibrous intimal thickening. This fibrous cap (FC) is mainly composed of collagens, elastin, and proteoglycans (Shah, 1996). Later, or in conjunction with this growth, an underlying lipid core (LC) may develop. So-called vulnerable or unstable plaques (UP) characteristically have a thin FC, a prominent LC, and abundant macrophages. In contrast stable plaques (SP) have a thicker FC, less voluminous LC, fewer macrophages, and more smooth muscle cells (SMC) (Davies et al., 1993). Disease progression sometimes results in a thinning of the FC that occurs largely due to the dissolution of the extracellular matrix (ECM). By this process an UP can evolve from a previously SP. It is this stage of lesion development where the plaque is considered extremely vulnerable to sudden rupture, an event that is frequently accompanied by thrombus formation leading to vascular occlusion and death (Libby, 1995).

The matrix metalloproteinase (MMP) family consists of more than twenty-four members of zinc endopeptidases that target a wide variety of substrates, including many of the ECM components that make up the arterial wall. MMP-1 and -8 are collagenases with the ability to cleave the major fibrillar collagens. MMP-2 and -9 are gelatinases that have the ability to digest collagen, and elastin. MMP-3 is a stromelysin that can degrade a wide variety of substrates including proteoglycans, collagen, and decorin. Belonging to the matrilysins, MMP-7 has a broad range of substrates including collagen and laminin. MMPs are secreted by a variety of cells such as endothelial cells (EC), smooth muscle cells (SMC) as well as cells involved in the inflammatory cascade (Keeling BW et al., 2005). MMPs have been associated with a wide variety of normal physiological processes (wound healing, skeletal formation) as well as pathological events (arthritis, aneurysm, atherosclerosis) (Lemaitre et al., 2006). Recent studies suggest that MMPs play an important role in the early vascular remodeling that accompanies the progression from the initial intima media thickening to the development of a large atheromatous plaque and ultimately the erosion of the ECM of the FC that contributes to plaque destabilization and rupture (Galis ZS and Khatri J, 2002). The proteolytic activities of MMPs are tightly regulated by a variety of endogenous inhibitors, including tissue inhibitors of metalloproteinases (TIMPs) (Nagase and Woessner, 1999). TIMP-1, the most prevalent of the TIMPs, inhibits most of the active MMPs. It has been proposed that an imbalance between MMPs and their respective inhibitors (TIMPs) may constitute an etiologic factor for cardiovascular disease. Relevant to this is the finding that MMP/TIMP ratios rather than MMP levels alone have sometimes been best correlated with arterial pathologies

Peripheral blood levels of MMP-2 and -9 were increased in patients with acute coronary syndrome (Kai H et al., 1998). In a recent study of premature coronary disease patients, plasma levels of MMP-2, MMP-3, MMP-9, TIMP-1, and TIMP-2 were measured (Noji et al., 2001). Significant differences were found in all MMPs and TIMPs between patients and controls. Increased expression of MMP-1 has been

found in vulnerable atherosclerotic plaques compared to non-lesion areas of the vessel (Galis et al., 1994). Furthermore, adenovirus-mediated overexpression of TIMP-1 in atherosclerosis susceptible apoE-deficient mice significantly reduced atherosclerotic lesions (Rouis et al., 1999).

In a study of carotid endarterectomy (CEA) patients, whose plaques were classified as stable or unstable, and in age-matched healthy volunteers, the plasma concentration of MMP-1, -2, -3, and -9, and TIMP-1 and -2 were analyzed (Sapienza et al., 2005). Pre-operative plasma levels of MMPs were higher from patients with UP compared to SP. Conversely TIMP-1 and -2 were lower in patients with UP compared to SP. The MMPs were lower in healthy volunteers than patients with either SP or UP while TIMP-1 and -2 levels were higher in healthy volunteers compared to those affected with UP, but similar to those with SP. In another of the few direct studies of human arterial tissue, MMP-2 and-9 and TIMP-1 and -2 were localized and quantified in CEA tissues of normal and atherosclerotic regions (Choudhary et al., 2006). The abundance of both MMPs was greater in plaque than in normal segments, but MMP-9 dominated. In normal areas both MMPs were lower but MMP-2 dominated. The authors also found an accumulation of MMPs at hemorrhagic sites. Within plaques, TIMPs were less abundant in calcified plaques and more abundant in fibrotic and necrotic segments. In another study of human carotid atherosclerotic plaque specimens, increased levels of MMP-1 and MMP-13 were detected in atheromatous (UP) compared to fibrous (SP) lesions (Sukhova et al., 1999).

Although a significant number of research studies indicate a role for the MMPs and TIMPs in the etiology of atherosclerosis, there are relatively few large population-based studies investigating the relationship between circulating MMPs and TIMP levels and the severity of atherosclerosis. To date, there are even fewer comprehensive studies such as the current one involving the measurement, correlation, and inter-relationships of multiple MMPs and TIMP-1 with one another and with specific parameters of carotid artery plaque architecture. Therefore, we propose to investigate the association of circulating levels of MMPs and TIMP-1 with carotid artery plaque characteristics in the ARIC carotid MRI cohort.

References:

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5. Main Hypothesis/Study Questions:

Increased MMP-1, -2, -3, -7, -8, -9 (MMPs) and decreased TIMP-1 plasma levels are associated with:

A) Increased atherosclerosis as measured by increased wall thickness or total wall volume.

B) Decreased fibrous cap thickness among those participants with a maximum wall thickness >= 1.5 and the presence of a lipid core.

<u>Secondary Hypotheses:</u>

Plasma MMP profile(s) are associated with carotid artery characteristics. (A scoring index may be used for these analyses based upon ranking of individual MMPs and TIMP-1 by quartiles, for example).

I. MMPs and TIMP-1 are associated with unstable plaque (*i.e. thin fibrous cap with large lipid core) compared to stable plaque, among those participants with plaque (*We will explore the definition of unstable plaque based upon measurements of fibrous cap thickness or volume and lipid core volume or area)
II. MMP/TIMP-1 ratios are better predictors of carotid artery characteristics than MMPs or TIMP-1 plasma levels.
III. The production and secretion of MMPs and TIMP-1 are co-regulated so that plasma MMPs and TIMP-1 levels are associated with one another.
IV. Plasma MMPs and TIMP-1 are associated with the inflammatory marker hsCRP and traditional lipid risk factors.
V. Increased plasma TIMP-1 levels are inversely correlated with carotid plaque calcification.

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 methodological limitations or challenges if present).

This study has a cross-sectional design. Subjects for the current study were selected from the Atherosclerotic Risk in Community (ARIC) cohort by first administering an ultrasound Doppler of the carotid artery to measure intima-media thickness (IMT). 1200 subjects in the >85th percentile of IMT were chosen as carotid artery disease (CAD) subjects and 800 individuals randomly samples from the remainder of those <85 percentile were selected as controls (C). Gadolinium-enhanced MRI was used to measure and quantify specific carotid artery characteristics. Circulating levels of MMP-1, -2, -3, -7, -8, and -9, and TIMP-1 were measured in EDTA plasma from visit 5.

MRI variables will include:

GDSICA-TOTALWALLVOLUME
GDSICA-MAXWALLTHICK-MAXCORE
MEAN-CAP-THICKNESS-2ADJACENT
MEAN-MIN-CAP-THICKNESS-2ADJACENT
LUMENAREA-MAXWALL
VESSELWALLAREA-MAXMEANWALL
GDSICA-MAXCALCIUMAREA
GDSICA-TOTALLIPIDCOREVOLUME
GDSICA-MAXLIPIDCOREAREA
LIPIDCORE

Other covariates will include age, gender, BMI, diabetes, smoking (never, current, and former), systolic and diastolic blood pressure, total cholesterol, and current drug regimen (lipid lowering, anti-hypertensive, anti-diabetic, anti-inflammatory).

All analysis is based on methods appropriate for stratified random sample methods.

In particular, all analyses are weighted by the inverse of the sampling fractions in the 8 sampling strata (4 field centers X 2 IMT groups) The association between MRI variables and MMPs and TIMP-1 will be analyzed by linear regression for continuous MRI variables and logistic regression for categorical MRI variables, with the MRI variables as the dependent variables, adjusted first for Model 1 (basic model):age, sex, and race, and then additionally for other covariates, including Model 2: Model 1 + total cholesterol, HDL-C, and triglycerides, and Model 3: Model 2 + smoking, BMI, blood glucose, blood pressure, use of blood pressure-lowering medication, lipid-lowering medication, aspirin and anti-arthritis medication, diabetes medications, and CRP. The association between MMPs and TIMP-1 and fibrous cap thickness will be analyzed both as a continuous variable and categorized as "thin" and "thick".
For adjustment for standard risk factors, outside of age, sex, and race, the analysis will consider both concurrent (cross-sectional) measures of risk factors as well as cumulative exposure or rate of change of exposure. The cumulative exposures will be determined for continuous variables as the area under the curve of exam-specific values plotted versus exam time, divided by time between first and last exam. This can be interpreted as the estimated mean daily value over the period. For dichotomous risk factors the cumulative indicator is the proportion of time exposed. For the continuous variables we will calculate the rate of change over the period as the person-specific slope from a random coefficients linear model.

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? ____ Yes _X___ 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"?
____ 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)?
MS#1204. MMP2 genetic variation influences measures of fibrous cap thickness: The Atherosclerosis Risk in Communities Carotid MRI Study. Lead author: Kelly A. Volcik

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* _2004.11__)

___ 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.