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Association of Circulating Matrix Metalloproteinases with Carotid Artery Characteristics: The ARIC Carotid MRI Study

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Abstract

Objective—To examine the relationship of plasma levels of matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinase-1 (TIMP-1) with carotid artery characteristics measured by magnetic resonance imaging (MRI) in a cross-sectional investigation among Atherosclerosis Risk in Communities (ARIC) Carotid MRI Study participants.

Methods and Results—A stratified random sample was recruited based on intima-media thickness (IMT) from a previous ultrasound examination. A high-resolution gadolinium-enhanced MRI exam of the carotid artery was performed in 2004–2005 on 1,901 ARIC cohort participants. Multiple carotid wall characteristics including wall thickness, lumen area, calcium area, lipid core and fibrous cap measures were evaluated for associations with plasma MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9 and TIMP-1.

Plasma MMP-1, MMP-3, and MMP-7 were significantly higher among participants in the high IMT group compared to those in the low IMT group. Normalized wall index was independently associated with MMP-3, MMP-7, and TIMP-1. MMP-7 was positively associated with carotid calcification. Mean fibrous cap thickness was significantly higher in individuals with elevated TIMP-1 levels. In addition, TIMP-1 was positively associated with measures of lipid core.

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Conclusions—Circulating levels of specific MMPs and TIMP-1 were associated with carotid wall remodeling and structural changes related to plaque burden in the elderly.

Keywords

Atherosclerosis; Carotid; MRI; MMP; TIMP-1; IMT; Luminex; Multianalyte Profiling

Atherosclerosis, the major cause of cardiovascular disease and stroke, is a complex inflammatory process that is characterized by the formation of raised arterial lesions eventually resulting in the narrowing of the lumen.[1] Of individuals who suffer an adverse cardiovascular event, as many as 50% have no apparent established traditional risk factor present.[2] Recent studies suggest that matrix metalloproteinases (MMPs) play an important role in the early vascular remodeling that accompanies the progression from the initial intima-media thickness (IMT) to the development of a large atheromatous plaque and ultimately to the erosion of the extracellular matrix (ECM) of the fibrous cap which contributes to plaque destabilization and rupture.[3] The MMP family consists of more than 24 members of zinc endopeptidases which target a wide variety of substrates, including most of the ECM components that make up the arterial wall.[4] MMP-1 and MMP-8 are interstitial collagenases with the ability to cleave the major fibrillar collagens. MMP-2 and MMP-9 are gelatinases that have the ability to digest vascular smooth muscle cell (SMC) basement membranes, 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), vascular SMCs, as well as cells involved in the inflammatory cascade.[5] The proteolytic activities of MMPs are tightly regulated, and a variety of endogenous inhibitors, including tissue inhibitor of metalloproteinase (TIMPs), are involved.[4] TIMP-1, the first discovered and most widely studied of the TIMPs, inhibits most of the active MMPs.

Systemic MMP or TIMP levels may constitute markers of the atherosclerotic process occurring in the vascular tree that are independent of traditional risk factors.[6] Adenovirus-mediated over-expression of TIMP-1 in atherosclerosis susceptible apo E-deficient mice significantly reduced atherosclerotic lesions.[7] In one of the few direct studies of human arterial tissue, MMP-2, MMP-9, TIMP-1 and TIMP-2 were localized and quantified in carotid endarterectomy (CEA) tissues of normal and atherosclerotic regions.[8] The abundance of both MMPs were greater in plaque than in normal segments. TIMPs were less abundant in calcified regions and more abundant in fibrotic and necrotic segments. In a study utilizing both human autopsy and surgical specimens, increased expression of MMP-1 was found in vulnerable atherosclerotic plaques compared to non-lesion areas of the vessel.[9] In a small study of 33 patients, peripheral blood levels of MMP-2 and MMP-9 were increased in patients with acute coronary syndrome.[10] In a recent case-control study of premature coronary disease in 53 consecutive male patients, plasma levels of MMP-2, MMP-3, MMP-9, TIMP-1, and TIMP-2 were measured, and significant differences were found in all MMPs and TIMPs between patients and controls.[11]

Although several epidemiological studies have used carotid ultrasound to measure carotid IMT and assess presence or absence of plaque, carotid ultrasound, as performed in ARIC and other studies, cannot reliably quantify plaque and wall volumes, lumen area, or vascular remodeling. MRI can extend the basic ultrasound measurements to assess plaque burden and vascular remodeling. For example, MRI-derived variables such as “normalized wall index” (NWI) can provide a measure of plaque burden which takes into account inherent differences in wall area among vessels of differing diameters. Additionally, MRI provides

the opportunity to measure, albeit with less accuracy, other structural components such as the fibrous cap, lipid core, and calcification.

To date, we are not aware of any comprehensive studies such as the current one that has explored the relationships between multiple MMPs and TIMP-1 and specific parameters of carotid artery wall architecture as measured by MRI. Therefore, we have investigated the association of circulating levels of MMPs and TIMP-1 with carotid artery wall characteristics in the large biracial Atherosclerosis Risk in Communities (ARIC) Carotid Magnetic Resonance Imagery (MRI) Study cohort.

METHODS

Study Design and Study Participants

The ARIC Study is a prospective investigation of atherosclerosis involving 15,792 African American and Caucasian men and women aged 45 to 64 years at recruitment (1987–1989). Participants underwent a baseline and up to 3 follow-up visits through 1998. A detailed description of the ARIC study design and methods has been published elsewhere.[12] The ARIC Carotid MRI Study was conducted between 2004 and 2005 as a cross-sectional sub-study. The participants were selected from the larger ARIC Study cohort based on results of the last ultrasound examinations (Visits 3 and 4, 1993–1998). In order to increase the prevalence of informative plaques while maintaining the ability to make population-based inferences, a stratified sampling plan was used. The goal was to recruit 1,200 participants with high values of maximum carotid artery wall thickness (maximum over 6 sites: common, bifurcation, internal carotid arteries of the left and right sides) and 800 participants randomly sampled from the remainder of the cohort below this cut point. This carotid artery IMT cut point was determined for each field center based on the IMT distribution specific to that site, and field-center-specific cut points of carotid IMT were adjusted over the recruitment period to approximately achieve this goal.

Recruitment lists were provided to the field centers, which sampled from above and below the IMT cut point as indicated by the sampling plan. Ineligibility criteria for the Carotid MRI Study included standard contraindications to the MRI exam or to the contrast agent, carotid revascularization on either side for the low IMT group or on the imaging-selected side for the high IMT group. Of the total of 4,307 persons who were contacted and invited to participate in the study, 1,404 refused, 837 were ineligible, and 2,066 participated. The overall recruitment rates were 40%, 48%, 51%, and 53% of all persons contacted from the 4 centers, with little difference in rates between high and low IMT groups. In addition, selected participants also received in-person interviews and a physical examination.

Of these 2,066 ARIC cohort members who participated in the Carotid MRI Study, 1,901 had a complete MRI exam, and 1,769, of the 1,901, had a sufficient quality of MRI scans and adherence to the MRI protocol to be included. Additionally, 91 participants with 1 or more missing values for any of the MMP or TIMP-1 measurements were excluded leaving 1,678 subjects in the final analyses. The average age of these 1,678 participants was 70 years and included 1,304 Caucasians (632 women and 672 men) and 374 African-Americans (220 women and 154 men).

Participant Examination

Protocols for lipoprotein, fasting glucose, blood pressure (BP), and height and weight measurements were identical at the ARIC cohort baseline examination and the Carotid MRI Sub-study examination conducted 18 years later. Cigarette smoking was ascertained from an interview and categorized as “current,” “former,” or “never.” Prescription drug use was obtained by self-report. Fasting blood samples were collected and assayed for total

cholesterol and high-density lipoprotein (HDL) cholesterol. Low-density lipoprotein (LDL) cholesterol was calculated according to the Friedewald formula. We assessed C-reactive protein (CRP) levels by the immunoturbidimetric CRP-Latex (II) high-sensitivity assay from Denka Seiken (Tokyo, Japan) performed according to the manufacturer's protocol using an Olympus AU400e automated chemistry analyzer (Olympus America Inc., Melville, NY). Resting BP was determined 3 times using a random-zero sphygmomanometer, and the mean of the last 2 measurements was used. Hypertension was defined as a systolic BP ≥140 mm Hg, diastolic BP ≥90 mm Hg, or use of antihypertensive medication during the previous 2 weeks. Diabetes mellitus (DM) was defined as a fasting glucose level of at least 126 mg/dL, a non-fasting glucose level of at least 200 mg/dL, or a self-reported history of physician-diagnosed DM or treatment for DM. Previous history of cardiovascular disease (CVD) included adjudicated myocardial infarction (MI), coronary heart disease (CHD) death, stroke, and/or revascularization procedure.[13] The study was approved by the institutional review committees of all participating centers and all participants provided informed consent.

MRI Protocol

MRI studies were performed on a 1.5T scanner (GE Medical Systems, Milwaukee, WI at 3 field centers; Siemens Medical Solutions, Ehrlangen, Germany at one field center) equipped with a bilateral 4-element phased array carotid coil (Machnet, The Netherlands). A 3-dimensional time-of-flight magnetic resonance angiogram (MRA) was acquired through both carotid bifurcations. Detailed black blood MRI (BBMRI) images were then acquired through the extracranial carotid bifurcation known to have a thicker maximum wall by the most recent ultrasound study, unless the contralateral carotid bifurcation wall appeared thicker on the MRA to the technologist. BBMRI imaging was achieved using a cardiac-gated, 2-dimensional double inversion recovery fast spin echo sequence with the inversion time set to suppress the signal of blood. Each participant received an intravenous injection of gadodiamide (Omniscan, GE Amersham), 0.1 mmol/kg body weight, using a power injector. Sixteen transverse T1-weighted BBMRI images (acquired resolution, $0.51\times0.58\times2mm^3$; total longitudinal coverage, 3.2cm) were oriented perpendicular to the vessel and centered at the thickest part of the internal or common carotid artery wall. These 16 slices were acquired 5 minutes after the injection through the thicker carotid artery using a 2-dimensional double inversion recovery fast spin echo sequence with the inversion time set to suppress the signal of the contrast-enhanced blood pool.

Image Analysis

MRI images were analyzed by 7 specially trained readers, blinded to the clinical and laboratory characteristics of the study population. All exams were assessed for image quality and protocol adherence, and exams that failed were not analyzed.

Slices were numbered 1 through 16 from proximal to distal, and only 8 slices, centered around the slice with the thickest wall, were analyzed. The plaque components were analyzed on the postcontrast BBMRI series based on the ability of gadolinium enhancement to delineate and enable quantitative size measurements of the fibrous cap,[14] and contours were drawn to delineate the outer wall, lumen, lipid core, and calcification. The total vessel area included lumen, intima, media and adventitia. Wall area was calculated as the difference between total vessel area and lumen area. The derived MRI variable Normalized Wall Index (NWI) was calculated by dividing the wall area by the total vessel area. Reliability coefficients (R) for the aforementioned MRI variables were obtained from an internal reliability study and have been published previously.[15] Generally, reliability for most MRI variables varied from excellent ($R>0.75$) to fair-to-good ($0.4<R<0.75$). However, the limited resolution of the 1.5T MRI may have contributed to the lower reliability of

measurements related to relatively small structural features such as the fibrous cap thickness. Although, reliability based on repeated readings was fair to good for cap thickness measures (R=0.60), reliability based on repeated scans was poor for cap thickness (R=0.38). A detailed description of the image analysis methods for the ARIC carotid MRI study has been published previously.[15]

Multianalyte profiling (MAP) assay

Fluorokine MAP allows the simultaneous measurement of multiple biomarkers from small sample volumes. In the current study, 6 MMP analytes were measured utilizing an assay from R&D Systems, Inc. (Minneapolis, MN) according to manufacturer's protocol with the following minor modifications. Due to differences in the plasma levels of the MMPs and the sensitivity of the assay, two different dilutions were utilized. Panel A measured MMP-1, MMP-7, and MMP-8 which required a 2-fold dilution, while Panel B measured MMP-2, MMP-3, and MMP-9 which required a 10-fold dilution. MMP control materials were obtained from R&D, and appropriate mixtures for Panel A and Panel B were run for each assay. Duplicates were run for all test samples, controls, and standards. Bio-Plex Manager 4.1 software (BioRad Labs) was used for data acquisition. TIMP-1 was measured with a solid-phase sandwich ELISA technique (R&D Systems) according to the manufacturer's protocol. Reliability coefficients for MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, and TIMP-1 were 0.87, 0.61, 0.66, 0.82, 0.79, 0.78, and 0.60, respectively based on blinded replicate pairs of 60 participants. Intra- and inter-assay coefficient of variation for MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, and TIMP-1 were 7.0 and 8.6%, 3.4 and 5.9%, 3.9 and 6.2%, 5.1 and 12.6%, 7.6 and 11.5%, 3.6 and 5.6%, and 4.7 and 6.9%, respectively. MMPs were measured as the total of all forms including pro-, mature-, and TIMP-complexed-forms; total TIMP-1 was also measured as both the free- and MMP-complexed-forms.

Statistical Analysis

All analyses were based on methods appropriate for a stratified random sampling. In particular, all analyses were weighted by the inverse of the sampling fractions in the 8 sampling strata (4 field centers × 2 IMT groups). The sampling fractions were based on those persons screened for participation. Those who actually participated were analyzed as a domain for calculating variances and confidence intervals of estimators. Analyses were conducted using SAS version 9.1 for descriptive statistics or SUDAAN for domain analysis. Finite population correction factors were not applied. Tests of differences in weighted means or proportions between groups were from weighted linear or logistic regression models that accounted for the sampling. Adjusted means and proportions by sub-group of interest were calculated using SUDAAN REGRESSION for continuous variables and LOGISTIC for dichotomous variables, with predicted values calculated as sample means of the adjusting variables. Wall thickness and wall volume were analyzed in the full data set of 1,678 participants. Due to the resolution constraints of the MRI scan, we restricted consideration of lipid core to the 1,156 participants whose maximum wall thickness was ≥1.5 mm (weighted percentage=62%). Only 4 lipid cores were excluded using this cut point. Measures of lipid core volume and area, and fibrous cap thickness were analyzed as continuous variables among those 554 participants with a lipid core. An additional analysis considered correlates of lipid core presence as a dichotomous variable. Standardized regression coefficients are presented for linear and logistic regression models, standardizing by 1-standard deviation of exposure and outcome (for continuous outcomes) with adjustment for age, race, and gender.

RESULTS

We investigated the relationship between circulating levels of MMPs and TIMP-1 and a number of traditional cardiovascular risk factors, as shown in Table 1. With the exception of MMP-9, all measured MMPs and TIMP-1 were positively associated with age. Interestingly, MMP-1 and MMP-2 were negatively associated with LDL-cholesterol. We also found that circulating levels of MMP-1, MMP-8, MMP-9, and TIMP-1 were negatively associated with HDL-cholesterol. Furthermore, MMP-8 and TIMP-1 were positively related to body mass index (BMI) as well as the inflammatory marker CRP. Elevated plasma levels of MMP-7 and TIMP-1 were associated with a higher incidence of hypertension, and MMP-1 and MMP-7 were similarly increased with incident diabetes. Individuals who used statins were more likely to have elevated plasma levels of MMP-1, MMP-2 and MMP-7. No obvious relationships were observed between circulating levels of MMP-s/TIMP-1 and smoking status or family history of CVD. However, individuals with a prior self-history of CVD were more likely to have elevated (3rd tertile) MMP-3 plasma levels.

Supplemental Table 1 shows the age-adjusted, race- and gender-specific plasma levels of MMPs and TIMP-1. Plasma levels of MMP-2 and MMP-3 were significantly higher in men than women of either race. In contrast, plasma levels of MMP-7 were lower overall in men than women. Furthermore, African Americans had increased plasma levels of MMP-7, MMP-8, and MMP-9 when compared to those in Caucasians. Strikingly, circulating levels of MMP-9 were elevated nearly 2-fold in African Americans. In addition to circulating levels of MMPs, a number of traditional cardiovascular risk factors also differed significantly by ethnicity and gender. C-reactive protein (CRP) was increased in women compared to men and higher overall in African Americans than Caucasians. The prevalence of both hypertension and diabetes was also higher overall in African Americans than Caucasians.

The simultaneous measurement of a number of different MMPs in this large cohort study enabled us to investigate potentially significant inter-relationships, which are summarized in Table 2. Generally, correlations between the various MMPs and TIMP-1 were low to moderate (Pearson R varied from −0.008 [between TIMP-1 and MMP-9] to 0.352 [between TIMP-1 and MMP-7]). TIMP-1 showed significant positive correlations with all MMPs measured with the exception of MMP-9. In contrast, MMP-9 did not correlate with any of the other MMPs measured except for a significant positive correlation with MMP-8.

The weighted means or proportions of traditional cardiovascular risk factors and MMPs and TIMP-1, adjusted by age, race, and gender are shown in Table 3. The results are stratified into high and low IMT groups, based upon ultrasound exams for participant selection as described in the “Methods” section. Systolic BP, BMI, smoking (both current and former), hypertension, and lipid-lowering medication (statin) use were all greater in the high IMT group. Furthermore, circulating levels of MMP-1, MMP-3, and MMP-7 were significantly elevated in the high IMT participants.

We examined the relationship of individual MMPs and TIMP-1 with various carotid wall measures as determined by MRI (Table 4). Circulating levels of MMP-7 (p=0.0394) and TIMP-1 (p=0.0192) were positively and statistically significantly associated with total wall volume after adjustment for major cardiovascular risk factors. In order to examine possible associations of circulating levels of MMPs and TIMP-1 with plaque burden, we included the derived MRI variable “normalized wall index,” or NWI, in our analysis as previously described by Saam et al.[16] NWI is calculated as the wall area divided by the total vessel area (wall area + lumen area). NWI takes into account inherent differences in wall area among vessels of differing diameters and therefore may provide a more accurate measure of plaque

burden than measurement of stenosis alone.[17] Plasma levels of MMP-3, MMP-7, and TIMP-1 were positively associated with NWI after adjustment for major cardiovascular risk factors. To further investigate the relationship of MMPs and TIMP-1 with various carotid MRI variables, we focused our analyses on those participants who had a maximum carotid wall thickness of greater than 1.5 mm and presence of atherosclerotic plaque as determined by the presence of lipid core (Table 5). Regression analysis showed that circulating levels of TIMP-1 were independently and positively associated with fibrous cap thickness in a fully adjusted model (p=0.0439). Additional analysis in which fibrous cap thickness was treated as a dichotomized variable (i.e. above or below the weighted median) showed that individuals with plasma TIMP-1 levels in the 3rd tertile had an OR of 1.42 (95%CI; 0.79 – 2.55) for increased fibrous cap thickness compared to those individuals with TIMP-1 levels in the 1st tertile.

Plasma levels of MMP-1 and MMP-7 were positively associated with carotid artery calcification, although the association for MMP-1 did not quite reach significance at the p<0.05 level. Interestingly, MMP-9 showed a moderate inverse association with carotid artery calcification. Both MMP-9 (p=0.0062) and TIMP-1 (p=0.0146) levels were associated with measures of lipid core, including maximum lipid core area and maximum lipid core volume. However, MMP-9 and TIMP-1 were significantly elevated in the 2nd tertile only. Additional analyses showed that circulating levels of TIMP-1 were associated with increased odds for presence of lipid core in a model adjusted for age, gender, and race, although odds were somewhat attenuated in a fully adjusted model (Table 6).

DISCUSSION

Plasma levels of MMPs and TIMP-1 were associated with a number of conventional cardiovascular risk factors (Table 1). In particular, we found positive associations with age for most MMPs and TIMP-1, with the exception of MMP-9. In one of the few studies designed to investigate the effect of age on MMPs and TIMPs, a small cross-sectional study of 27 asymptomatic individuals aged 20–90 examined MMP-2, MMP-7, MMP-8, MMP-9, and TIMP-1, TIMP-2, and TIMP-4 plasma concentrations.[18] The authors reported increased levels of MMP-2, MMP-7, and all TIMPs, and reported no change in MMP-8 levels with age, the latter result being the only deviation from the current finding. Plasma levels of MMP-8 and TIMP-1 were positively associated with BMI as well as the inflammatory marker, CRP. Furthermore, hypertensive subjects and diabetics were more likely to have elevated levels of MMP-1, MMP-7 and TIMP-1. Overall, our findings show that circulating levels of MMPs and TIMP-1 were associated with an adverse cardiovascular risk profile, and therefore, are supportive of the current belief that MMPs play a critical role in the etiology of atherosclerosis and CVD.

The ARIC Carotid MRI study also provided a unique opportunity to examine the effect of race and gender on plasma levels of MMPs and TIMP-1 in a large biracial population. We found that plasma levels of MMP-2 and MMP-3 were significantly increased, and MMP-7 was decreased in men compared to women (Supplemental Table 1). A similar finding was observed in a case-control study of 387 myocardial infarction patients in which the MMP-3 serum concentrations for both case and control subjects were almost double for men compared to women.[19] Furthermore, in our biracial study cohort, MMP-7, MMP-8, and MMP-9 levels were elevated in African Americans compared to Caucasians.

A number of studies have shown that possible pleiotropic effects of statins may involve the down-regulation of MMP expression. Cerivastatin inhibited inducible MMP-1, MMP-3, and MMP-9 secretion from human SMCs.[20] In a study of 32 patients with CAD, 14 weeks of simvastatin treatment significantly reduced plasma MMP-9 concentration.[21] It has been

hypothesized that statins may elicit some of their atheroprotective functions by decreasing MMP levels thus leading to stabilization of the plaques with or without lesion regression. Our results, which show that MMP-1 and MMP-2 were negatively associated with LDL-cholesterol and individuals with elevated plasma levels of MMP-1 and MMP-2 were more likely to be on statin therapy (Table 1), may appear to contradict the findings from these previous studies. However, our findings may be confounded because high-risk patients (i.e., those with coronary heart disease) were more likely to be taking a statin. In addition, it is important to note that the ARIC study was not designed to investigate possible effects of statin therapy on circulating MMP levels.

We investigated the inter-relationships among MMPs and TIMP-1 plasma concentrations (Table 2). Significant correlations may result from coordinated gene expression or could represent a commonality of the tissues and cell types responsible for production of the MMPs that are mutually up- or down-regulated in response to shared agonists or antagonists.[22] We found MMP-8 and MMP-9 levels were correlated with one another ($r=0.214$, $p<0.0001$). A significant relationship between MMP-8 and MMP-9 was also found in a study of CEA specimens,[23] and strong correlations between those MMPs were noted in a study of adolescents.[24] MMP-8 and MMP-9 are both synthesized by differentiating granulocytes, stored in circulating neutrophils, and released following neutrophil activation,[25] a mutuality that could partially explain their association. TIMP-1 was significantly positively correlated with all MMPs except MMP-9; among these significant correlations, the association with MMP-8 was the weakest. The strongest correlation ($r=0.352$) was found between TIMP-1 and MMP-7 suggesting that their regulation may be closely linked. In the present study, both MMP-7 and TIMP-1 concentrations were significantly associated with total wall volume, maximum wall thickness at the lipid core, and NWI suggesting shared functional associations possibly related to their correlation.

Circulating levels of MMP-1, MMP-3, and MMP-7 were elevated among participants in the high IMT group as determined by ultrasound examination (Table 3). Furthermore, plasma MMP-7 and TIMP-1 levels were associated with increased total wall volume as determined by MRI, when modeled in tertiles. Normalized wall index (NWI) was associated with increased levels of MMP-3, MMP-7, and TIMP-1. Although NWI is recognized as an indicator of plaque burden, the measurement itself does not directly involve the lipid core or fibrous cap. The elevated plasma levels of MMPs and TIMP-1 associated with measures of increased carotid wall thickness may reflect their increased production leading to active arterial wall remodeling.

In addition to early atherosclerosis, MMPs have also been associated with plaque progression and development of the so-called vulnerable plaque.[10, 22] Although there are discordant reports concerning the effect of calcium on plaque stability, arterial calcification has often been associated with plaque vulnerability.[26, 27] Alternatively, arterial calcification may simply reflect overall atherosclerotic burden. In the present study, a significant association was found between plasma MMP-7 level and calcium area, suggesting a possible role for MMP-7 in arterial calcification.

Circulating levels of TIMP-1 were positively associated with presence of lipid core and lipid core area, as measured by MRI. Furthermore, elevated plasma TIMP-1 was associated with increased mean fibrous cap thickness. Taken together, our findings suggest that TIMP-1 may be involved in processes leading to thickening of the carotid artery wall and plaque progression into plaques with large lipid cores. However, the positive association of plasma TIMP-1 levels with mean fibrous cap thickness may point to a possible role for TIMP-1 in plaque stabilization. A number of in vitro studies as well as studies in animals and humans

have investigated the possible role of TIMP-1 in the etiology of atherosclerosis. However, to this date, there is only limited data available pointing to a possible causal link between TIMP-1 and atherosclerotic plaque progression. TIMP-1 has been shown to have a mitogenic effect on human aortic smooth muscle cells (SMC) suggesting that it may contribute to SMC proliferation in atherosclerosis.[28] In a prospective study of patients undergoing coronary angiography, elevated plasma TIMP-1 was an independent predictor of all-cause mortality and myocardial infarction.[29] Furthermore, increased circulating levels of TIMP-1 were associated with the presence of carotid artery lesions in a study of subjects with hyperlipidemia.[30] In contrast, we recently reported that plasma TIMP-1 levels were not predictive of incident coronary artery disease in a case-random cohort sample of the ARIC study.[31] However, it is plausible that differences in study design and population may be partly responsible for these seemingly contradictory findings. Alternatively, TIMP-1 could be up-regulated in response to a generalized MMP over-expression as a protective mechanism to prevent excessive ECM degradation and plaque destabilization.[22]

In the present study several factors could explain the larger number of significant associations observed between MMPs and TIMP-1 and variables related to general measures of the carotid wall compared to MRI variables specific to plaque. First, determinations such as wall thickness include the entire study group of 1,678 whereas measures of plaque characteristics are limited to only the 569 participants with a detectable lipid core, leading to differences in statistical power. Secondly, the resolution of the MRI limits the ability to characterize small plaques, restricting the study to the largest plaques found within the thickest arterial walls. Therefore, by truncating the distribution of lipid core volume and cap thickness, possible associations with MMP levels may have gone undetected in our study.

There are several limitations to the current study. First, MMPs/TIMP-1 were measured in the circulation rather than in the arterial wall itself. While it has been demonstrated that a number of different cell types present in atherosclerotic lesions are capable of MMP and TIMP synthesis, it is unlikely that circulating MMP levels solely reflect their release from atherosclerotic plaques. Instead, it is more likely that systemic MMP and TIMP levels reflect their general release from the vasculature, as well as interstitial compartments and other non-vascular sources. However, a number of investigators have reported associations between circulating MMPs and TIMP-1 levels and severity of atherosclerosis.[11, 12, 32, 33]

Secondly, total MMPs and TIMP-1 were measured, but it is the active forms of the MMPs that are responsible for their enzymatic functions. All of the MMPs studied here are secreted as the pro-enzyme, so this zymogen form is initially present before activation. If the synthesis and secretion of MMPs/TIMP-1 are increased in response to factors that regulate arterial remodeling events, then an increase in the total forms of the MMPs might be an indicator of increased remodeling activity. It would be most informative to measure the active forms in conjunction with the total forms of these proteases. The additional measurement of the active forms of the MMPs and their association with arterial wall characteristics would be desirable in future studies, since such data are lacking.

There are also strengths of the current study. First, the study group was selected from the well-characterized biracial population-based ARIC cohort, and to our knowledge, this is the largest population-based study investigating the relationship of several MMPs and TIMP-1 with quantitative measures of carotid wall components using a gadolinium contrast-enhanced MRI exam. Secondly, a standardized MRI protocol with central reading facility was utilized and extensive quality control data were collected allowing assessment of the reliability of the MRI measures as well as MMPs/TIMP-1 plasma concentrations.

A number of investigators have noted the importance of blood collection procedures especially concerning the measurements of circulating MMPs and TIMP-1.[34, 35] There is great discordance in the literature regarding the effects of MMPs and TIMPs, which could be due in part to blood collection procedures. Serum levels of a number of these analytes are influenced by the release of MMPs and TIMP-1 following degranulation of leukocytes and platelets during the ex-vivo blood clotting process in the specimen collection tube. The extent of this elevation varies for the specific analyte measured, but TIMP-1, MMP-1, MMP-8, and MMP-9 have been measured 5–20 times higher in serum than plasma. Since such elevated serum levels result in high background levels, it is generally agreed that plasma, the clinical specimen used in this study, is the preferred source for MMP and TIMP-1 measurement.[34] When comparing data from clinical studies related to circulating concentrations of MMPs attention should be given to the method of blood collection utilized.

In summary, our data indicate that circulating levels of MMPs and TIMP-1 are associated with distinct carotid wall characteristics. Additional studies are needed to investigate the relationship between MMPs and atherosclerosis burden.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Weighted means (SE) or proportion (SE) of Risk factors by weighted tertiles of each MMP/TIMP variable, and p-value for test for any differences between tertiles, adjusted by age, race, gender

Risk Factors*	MMP/TIMP Variables	1st Tertile	2nd Tertile	3rd Tertile	P-Value†
Age (years) (n=1678)	MMP-1	69.72 (0.282)	70.32 (0.276)	70.96 (0.307)	0.0149
	MMP-2	69.10 (0.283)	70.24 (0.277)	71.62 (0.287)	<0.0001
	MMP-3	69.13 (0.291)	70.32 (0.282)	71.51 (0.309)	<0.0001
	MMP-7	69.35 (0.287)	69.81 (0.278)	71.81 (0.279)	<0.0001
	MMP-8	69.65 (0.281)	70.24 (0.298)	71.08 (0.276)	0.0013
	MMP-9	70.61 (0.310)	70.07 (0.276)	70.26 (0.299)	0.4188
	TIMP-1	68.75 (0.238)	70.21 (0.291)	72.00 (0.300)	<0.0001
LDL Cholesterol (mg/dl) (n=1650)	MMP-1	117.65 (1.999)	114.99 (2.092)	108.55 (1.824)	0.0030
	MMP-2	117.57 (1.968)	114.09 (1.990)	109.94 (1.921)	0.0249
	MMP-3	109.80 (2.039)	116.31 (1.931)	115.53 (2.078)	0.0587
	MMP-7	114.87 (1.930)	115.30 (2.031)	111.43 (1.892)	0.3002
	MMP-8	115.38 (1.909)	115.16 (2.128)	111.00 (1.768)	0.1605
	MMP-9	114.84 (2.024)	116.65 (1.736)	110.10 (2.242)	0.0809
	TIMP-1	114.59 (1.983)	113.46 (1.899)	113.52 (2.084)	0.9051
HDL Cholesterol (mg/dl) (n=1678)	MMP-1	50.93 (0.762)	51.45 (0.830)	48.64 (0.710)	0.0176
	MMP-2	49.81 (0.745)	49.83 (0.748)	51.43 (0.820)	0.2395
	MMP-3	49.29 (0.875)	51.01 (0.717)	50.78 (0.825)	0.3128
	MMP-7	50.45 (0.738)	51.02 (0.770)	49.60 (0.811)	0.4344
	MMP-8	51.50 (0.806)	51.15 (0.747)	48.38 (0.725)	0.0039
	MMP-9	52.41 (0.817)	50.04 (0.752)	48.59 (0.791)	0.0034
	TIMP-1	52.29 (0.769)	51.14 (0.839)	47.63 (0.669)	<0.0001
BMI (kg/m^2) (n=1677)	MMP-1	28.34 (0.254)	28.24 (0.273)	28.56 (0.265)	0.6869
	MMP-2	28.91 (0.281)	28.18 (0.246)	28.04 (0.244)	0.0515
	MMP-3	28.66 (0.300)	28.43 (0.243)	28.04 (0.272)	0.3131
	MMP-7	28.17 (0.252)	28.52 (0.266)	28.44 (0.260)	0.6066
	MMP-8	27.28 (0.240)	28.52 (0.254)	29.37 (0.267)	<0.0001
	MMP-9	28.30 (0.283)	28.53 (0.250)	28.31 (0.260)	0.7866
	TIMP-1	27.50 (0.247)	28.34 (0.266)	29.31 (0.262)	<0.0001

Risk Factors*	MMP/TIMP Variables	1st Tertile	2nd Tertile	3rd Tertile	P-Value†
Log(CRP) (n=1669)	MMP-1	0.78 (0.050)	0.70 (0.052)	0.69 (0.058)	0.4397
	MMP-2	0.88 (0.055)	0.74 (0.050)	0.55 (0.052)	0.0001
	MMP-3	0.62 (0.056)	0.77 (0.053)	0.78 (0.056)	0.1138
	MMP-7	0.77 (0.054)	0.74 (0.053)	0.65 (0.050)	0.2420
	MMP-8	0.49 (0.049)	0.69 (0.052)	1.00 (0.053)	<0.0001
	MMP-9	0.73 (0.053)	0.63 (0.055)	0.80 (0.052)	0.0841
	TIMP-1	0.53 (0.054)	0.69 (0.049)	0.94 (0.053)	<0.0001
Hypertensive (n=1663)	MMP-1	60.0% (2.60%)	62.2% (2.59%)	66.1% (2.64%)	0.2657
	MMP-2	63.1% (2.64%)	59.2% (2.64%)	65.9% (2.55%)	0.1877
	MMP-3	60.3% (2.82%)	60.5% (2.64%)	67.3% (2.59%)	0.1294
	MMP-7	57.7% (2.65%)	60.5% (2.62%)	70.6% (2.54%)	0.0016
	MMP-8	59.8% (2.60%)	61.4% (2.61%)	67.3% (2.58%)	0.0994
	MMP-9	61.9% (2.65%)	61.5% (2.60%)	65.0% (2.78%)	0.6415
	TIMP-1	57.6% (2.62%)	63.0% (2.63%)	67.8% (2.59%)	0.0239
Diabetes (n=1661)	MMP-1	16.3% (1.77%)	22.4% (2.14%)	29.3% (2.54%)	0.0003
	MMP-2	21.3% (2.18%)	22.4% (2.17%)	23.1% (2.13%)	0.8403
	MMP-3	22.4% (2.43%)	21.7% (2.13%)	22.6% (2.27%)	0.9492
	MMP-7	17.0% (2.01%)	24.0% (2.27%)	25.8% (2.21%)	0.0133
	MMP-8	22.7% (2.23%)	19.3% (2.06%)	24.5% (2.21%)	0.2220
	MMP-9	20.9% (2.24%)	20.3% (2.14%)	25.2% (2.35%)	0.2731
	TIMP-1	20.0% (2.15%)	21.8% (2.16%)	24.8% (2.21%)	0.2940
Statin use (n=1656)	MMP-1	24.2% (2.12%)	34.7% (2.50%)	42.9% (2.77%)	<0.0001
	MMP-2	27.6% (2.39%)	39.4% (2.62%)	34.1% (2.46%)	0.0051
	MMP-3	37.7% (2.84%)	32.5% (2.48%)	31.2% (2.41%)	0.2441
	MMP-7	29.5% (2.45%)	33.5% (2.51%)	38.4% (2.56%)	0.0499
	MMP-8	30.1% (2.41%)	33.8% (2.52%)	37.5% (2.57%)	0.1196
	MMP-9	29.3% (2.37%)	34.6% (2.50%)	37.7% (2.79%)	0.0765
	TIMP-1	34.8% (2.54%)	32.4% (2.49%)	34.0% (2.49%)	0.7820
Self-History of CVD (Stroke or CHD) (n=1678)	MMP-1	9.7% (1.50%)	10.5% (1.46%)	12.2% (1.66%)	0.5373
	MMP-2	9.3% (1.58%)	9.1% (1.37%)	13.7% (1.67%)	0.0528
	MMP-3	10.5% (1.96%)	7.3% (1.23%)	13.8% (1.56%)	0.0037

Risk Factors*	MMP/TIMP Variables	1st Tertile	2nd Tertile	3rd Tertile	P-Value†
	MMP-7	9.4% (1.50%)	10.1% (1.52%)	13.0% (1.65%)	0.2333
	MMP-8	7.9% (1.36%)	12.6% (1.70%)	11.8% (1.49%)	0.0834
	MMP-9	9.2% (1.25%)	11.0% (1.60%)	12.7% (1.97%)	0.2958
	TIMP-1	9.7% (1.58%)	9.2% (1.44%)	13.3% (1.62%)	0.1138
Family History of CVD (Stroke or CHD) (n=1663)	MMP-1	56.3% (2.55%)	55.2% (2.63%)	58.5% (2.80%)	0.6784
	MMP-2	54.1% (2.73%)	56.2% (2.60%)	59.7% (2.61%)	0.3373
	MMP-3	55.2% (2.84%)	55.3% (2.66%)	59.3% (2.75%)	0.5090
	MMP-7	52.7% (2.67%)	57.7% (2.64%)	59.6% (2.61%)	0.1630
	MMP-8	53.5% (2.68%)	59.2% (2.61%)	57.3% (2.61%)	0.3136
	MMP-9	60.6% (2.68%)	54.4% (2.67%)	54.8% (2.82%)	0.2011
	TIMP-1	60.5% (2.58%)	53.8% (2.66%)	55.5% (2.66%)	0.1784
Current Smoker (n=1656)	MMP-1	6.5% (1.22%)	8.9% (1.53%)	7.7% (1.43%)	0.2851
	MMP-2	6.6% (1.26%)	9.8% (1.60%)	6.5% (1.27%)	0.4630
	MMP-3	7.8% (1.46%)	6.9% (1.25%)	8.2% (1.57%)	0.5827
	MMP-7	6.6% (1.31%)	7.4% (1.33%)	9.1% (1.50%)	0.5753
	MMP-8	7.1% (1.34%)	5.9% (1.23%)	10.0% (1.58%)	0.2233
	MMP-9	4.9% (1.18%)	7.6% (1.43%)	10.3% (1.72%)	0.1661
	TIMP-1	5.5% (1.14%)	8.5% (1.46%)	9.2% (1.58%)	0.3397

*n=number of included participants

†P-value for test of differences in means

TABLE 2

Weighted Pearson correlation coefficient between circulating levels of different MMPs and TIMP-1

	MMP-1	MMP-2	MMP-3	MMP-7	MMP-8	MMP-9	TIMP-1
MMP-1		0.044	0.150†	0.138‡	0.162*	−0.017	0.102‡
MMP-2			0.140‡	0.137‡	0.031*	0.017	0.262†
MMP-3				0.154‡	0.062‡	−0.025	0.133‡
MMP-7					0.022	0.037	0.352‡
MMP-8						0.214*	0.095‡
MMP-9							−0.008
TIMP-1							

*$P<0.05$

†$P<0.001$

‡$P<0.0001$

Table 3

Weighted mean (SE), or proportions of traditional risk factors and MMPs and TIMP-1 levels (mean [SE]) among MRI participants stratified by imputed IMT, adjusted by age, gender and race.

Characteristics	Low IMT group	High IMTgroup	P†
Number of included participants (N)	553	1125	
LDL Cholesterol (mg/dl)	113.97 (1.264)	113.24 (1.949)	0.7511
HDL Cholesterol (mg/dl)	50.43 (0.511)	49.94 (0.664)	0.5401
Systolic Blood Pressure (mmHg)	125.00 (0.643)	128.63 (1.031)	0.0028
Diastolic Blood Pressure (mmHg)	67.01 (0.349)	65.86 (0.519)	0.0645
BMI (kg/m2)	28.29 (0.166)	28.92 (0.219)	0.0154
Log (CRP)	0.72 (0.035)	0.75 (0.046)	0.5641
Current Smoker	7.0%	12.6%	0.0004‡
Former Smoker	41.9%	47.3%	
Hypertensive	60.9%	73.7%	<0.0001
Hypertensive medication use	53.8%	68.3%	<0.0001
Diabetes	21.6%	25.8%	0.1101
Diabetes Medication use	13.4%	17.1%	0.0959
Statin use	31.5%	46.6%	<0.0001
Anti-inflammatory Medication use	77.5%	83.3%	0.0146
MMP-1 (ng/ml)	0.172 (0.008)	0.210 (0.011)	0.0040
MMP-2 (ng/ml)	299.51 (4.30)	313.18 (6.35)	0.0849
MMP-3 (ng/ml)	6.30 (0.14)	7.30 (0.34)	0.0070
MMP-7 (ng/ml)	2.47 (0.057)	2.92 (0.10)	0.0001
MMP-8 (ng/ml)	0.452 (0.023)	0.457 (0.014)	0.8178
MMP-9 (ng/ml)	21.02 (0.39)	20.96 (0.44)	0.9261
TIMP-1 (ng/ml)	111.53 (1.07)	114.64 (1.57)	0.1103

*Data presented as mean (standard error of the mean) or percentages.

†P-value for test of differences in means.

‡P-value for test if 2 groups are different in current smoking OR former smoking. Thus, it is a 2-degrees-of-freedom test.

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Table 4

Weighted means (SE) or proportion (SE) of MRI variables by weighted tertiles of each MMP/TIMP variable, and p-value for test for any differences between tertiles, adjusted by age, race, gender, smoking, hypertension, CRP, diabetes, total cholesterol, and HDL cholesterol, on all participants

MRI Variables	CLDA Variables	1st Tertile	2nd Tertile	3rd Tertile	P-Value
Total Wall Volume (n=1678)*	MMP-1	4.065 (0.073)	4.131 (0.080)	4.210 (0.087)	0.4414
	MMP-2	4.118 (0.084)	4.088 (0.078)	4.195 (0.083)	0.6232
	MMP-3	4.120 (0.080)	4.038 (0.079)	4.245 (0.090)	0.2174
	MMP-7	3.984 (0.069)	4.193 (0.094)	4.231 (0.078)	0.0394
	MMP-8	4.126 (0.078)	4.136 (0.077)	4.140 (0.086)	0.9921
	MMP-9	4.150 (0.081)	4.216 (0.086)	4.034 (0.074)	0.2500
	TIMP-1	3.963 (0.075)	4.278 (0.092)	4.161 (0.076)	0.0192
Normalized Wall Index (NWI) (n=1678)*	MMP-1	0.432 (0.007)	0.432 (0.007)	0.450 (0.007)	0.1406
	MMP-2	0.438 (0.007)	0.435 (0.007)	0.441 (0.007)	0.8164
	MMP-3	0.431 (0.007)	0.426 (0.007)	0.457 (0.008)	0.0080
	MMP-7	0.426 (0.007)	0.438 (0.007)	0.450 (0.007)	0.0587
	MMP-8	0.433 (0.007)	0.438 (0.007)	0.444 (0.008)	0.5688
	MMP-9	0.433 (0.007)	0.443 (0.007)	0.437 (0.008)	0.5807
	TIMP-1	0.419 (0.006)	0.448 (0.007)	0.447 (0.007)	0.0040

*n=number of included participants

Table 5

Weighted means (SE) or proportion (SE) of MRI variables by weighted tertiles of each MMP/TIMP variable, and p-value for test for any differences between tertiles, adjusted by age, race, gender, smoking, hypertension, CRP, diabetes, total cholesterol, and HDL cholesterol, and restricted to participants with Max Wall Thickness >1.5 and Lipid Core=1.

MRI Variables	CLDA Variables	1st Tertile	2nd Tertile	3rd Tertile	P-Value*
Mean Fibrous Cap Thickness (mm) (n=544)*	MMP-1	0.682 (0.028)	0.642 (0.031)	0.700 (0.028)	0.3684
	MMP-2	0.678 (0.029)	0.673 (0.033)	0.678 (0.026)	0.9918
	MMP-3	0.682 (0.032)	0.683 (0.033)	0.668 (0.026)	0.9254
	MMP-7	0.684 (0.034)	0.640 (0.026)	0.701 (0.026)	0.2287
	MMP-8	0.673 (0.026)	0.652 (0.037)	0.700 (0.024)	0.5126
	MMP-9	0.663 (0.034)	0.664 (0.028)	0.702 (0.028)	0.6090
	TIMP-1	0.626 (0.024)	0.669 (0.032)	0.718 (0.027)	0.0439
Maximum Calcium Area (mm^2) (n=545)*	MMP-1	0.032 (0.003)	0.038 (0.004)	0.044 (0.006)	0.0642
	MMP-2	0.042 (0.009)	0.037 (0.003)	0.037 (0.004)	0.8757
	MMP-3	0.034 (0.003)	0.036 (0.004)	0.044 (0.006)	0.3264
	MMP-7	0.027 (0.002)	0.045 (0.008)	0.043 (0.004)	0.0002
	MMP-8	0.045 (0.008)	0.033 (0.003)	0.038 (0.004)	0.2209
	MMP-9	0.042 (0.006)	0.039 (0.004)	0.033 (0.003)	0.3015
	TIMP-1	0.035 (0.004)	0.042 (0.007)	0.038 (0.003)	0.4951
Maximum Lipid Core Area (mm^2) (n=554)*	MMP-1	0.102 (0.011)	0.115 (0.010)	0.109 (0.010)	0.6936
	MMP-2	0.098 (0.007)	0.110 (0.009)	0.115 (0.013)	0.3876
	MMP-3	0.110 (0.009)	0.097 (0.008)	0.115 (0.011)	0.3724
	MMP-7	0.099 (0.008)	0.121 (0.013)	0.105 (0.011)	0.3207
	MMP-8	0.112 (0.012)	0.101 (0.009)	0.111 (0.011)	0.7276
	MMP-9	0.101 (0.010)	0.1348 (0.013)	0.0922 (0.007)	0.0062
	TIMP-1	0.089 (0.008)	0.1325 (0.014)	0.0990 (0.008)	0.0146

*n=number of included participants

Table 6

Adjusted odds ratios for lipid core presence according TIMP-1 levels modeled in tertiles

	Model 1			Model 2		
Variable	**Odds Ratio**	**95% CI**	***P***	**Odds Ratio**	**95% CI**	***P***
TIMP-1						
2nd tertile vs. 1st tertile	1.49	1.02 – 2.17	0.039	1.37	0.93 – 2.01	0.113
3rd tertile vs. 1st tertile	1.54	1.05 – 2.24	0.026	1.41	0.95 – 2.10	0.092

Model 1: Adjusted for age, gender and race

Model 2: Adjusted for variables included in Model 1 and smoking, hypertension, diabetes, total cholesterol, HDL cholesterol, and CRP.