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Association of Carotid Artery Atherosclerosis with Circulating Biomarkers of Extracellular Matrix Remodeling: The Framingham Offspring Study

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Abstract

Objective—To relate circulating biomarkers of extracellular matrix (ECM) turnover to site-specific measures of carotid artery atherosclerosis on duplex ultrasound.

Background—Matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) regulate ECM remodeling, a key feature of atherosclerosis, and their circulating concentrations can be assayed. MMP-9, TIMP-1 and protocollagen-III n-terminal propeptide (PIIINP) may relate differentially to the severity of atherosclerosis at different carotid artery sites. However, data examining this premise are sparse.

Design/Methods—We related circulating MMP-9, TIMP-1 and/or PIIINP concentrations to carotid atherosclerosis on duplex ultrasound in1006 Framingham Offspring (mean age 58 years, 56% women) who attended a routine examination from 1995–1998. We used multivariable regression to relate MMP-9 (detectable versus undetectable), and TIMP-1 and PIIINP (age- and sex-specific quartiles) to internal carotid artery stenosis (>25%), and log-transformed common and internal carotid intima-media thickness (CC-IMT, IC-IMT, respectively).

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The authors report no conflicts of interest.

Results—Detectable MMP-9 was associated with carotid stenosis (OR 1.71, p=0.032) but not with IMT. Higher TIMP-1 was associated with carotid stenosis (OR for Q4 versus Q1-3, 1.63, p=0.022) and a higher IC-IMT (β 0.057 ±0.025, Q4 versus Q1-3, p=0.023). Higher PIIINP (Q4 versus Q1-3) showed a borderline association with carotid stenosis (OR 1.45 for Q4 versus Q1-3, p=0.095) but not with IMT. TIMP-1 was not associated with CC-IMT.

Conclusions—In our community-based sample of middle-aged to older adults, higher circulating biomarkers of matrix remodeling were associated with a greater prevalence of carotid stenosis, and subclinical atherosclerosis in the IC artery. Our findings are consistent with regional differences in matrix remodeling in the carotid artery.

Keywords

Carotid artery; atherosclerosis; matrix proteins; intima media thickness; stenosis

Introduction

Carotid artery stenosis and carotid intima-media thickness (IMT) are markers of atherosclerosis, and have been associated with cardiovascular events including stroke and myocardial infarction.(1) All phases of atherosclerosis in humans are characterized by remodeling of the extracellular matrix (ECM), a complex process in which the matrix metalloproteinases (MMPs) play a pivotal role.(2–7) The MMPs are a group of more than 20 endopeptidases capable of modifying most components of the ECM.(8) Most of the cells that have been implicated in the development of atherosclerosis have been shown to produce MMPs in carotid arteries, including macrophages,(9) mastocytes,(3) endothelial and smooth muscle cells.(9) The recent availability of high-sensitivity assays permits evaluation of circulating concentrations of MMPs and their inhibitors, the tissue inhibitors of matrix metalloproteinases (TIMPs).

The carotid bifurcation and internal carotid artery are sites with a higher predilection for the formation of atherosclerotic plaques. Thus, the initial atherosclerotic changes and the progression of the process may be particularly evident at these vascular locations. For instance, in the Atherosclerosis Risk in Communities (ARIC) Study, mean IMT varied across the internal carotid artery, the carotid bifurcation, and the common carotid artery.(10) In addition, prior studies have shown that rates of IMT progression are greatest at the internal carotid artery, followed by the bifurcation, and then the common carotid artery.(11–13) Indeed, the IMT progression rate was more than 30 times greater at the internal carotid compared to the common carotid artery in a prior report.(11) These site-specific differences are important because they may explain why relations of cardiovascular risk factors to carotid atherosclerosis vary at different carotid segments. In the Cardiovascular Health Study (CHS), baseline vascular risk factors were not related to progression of common carotid artery-IMT.(14) Another study showed an association of elevated LDL cholesterol and cigarette smoking with plaques at the carotid bifurcation, whereas higher systolic blood pressure and body mass index (BMI) were associated with common carotid artery plaques. (15) In addition, the predictive value of measures of atherosclerosis may vary according to the site of measurement. For instance, internal carotid IMT has been shown to be a better predictor of myocardial infarction compared to measures in the common carotid artery, whereas the combined measurement (common and internal carotid IMT) was better predictor of stroke in a prior study.(1)

The pathophysiology of site-specific changes is not entirely clear, but may involve segment-specific arterial remodeling, including that of the vascular extracellular matrix. Therefore, we evaluated the relation of plasma concentrations of select matrix biomarkers (including MMP-9, its inhibitor TIMP-1, and PIIINP, the N-terminal propeptide of protocollagen III that is a

product of degradation of Type III collagen) and site-specific measures of carotid atherosclerosis assessed via carotid ultrasound in a community-based sample.

Methods

Study Cohort

The Framingham Offspring Cohort was recruited in 1971, and includes subjects who are either children or the spouses of the children of the participants in the Original Framingham Heart Study. The members of the Offspring Cohort have been examined approximately every four to eight years since 1971. At the 6th examination cycle, 3377 attendees underwent carotid duplex ultrasound. Participants with available carotid ultrasound measurements and plasma biomarkers of ECM matrix turnover (MMP-9, TIMP-1 and/or PIIINP) constituted the sample for the present study; we excluded individuals with clinically evident stroke or dementia.

Sampling scheme—Only a subsample of attendees had plasma concentrations of matrix biomarkers measured because such assessment was performed to assess the biochemical correlates of left ventricular remodeling; therefore, the sampling scheme for the present investigation was based on the design of the echocardiographic study.(16) Briefly, participants were categorized into three groups based on their echocardiographic left ventricular (LV) measures: (1) participants with values of LV end-diastolic diameter (LVEDD) and wall thickness (LVWT) below the sex-specific medians; (2) group with increased LVEDD values greater than or equal to the sex-specific 90th percentile; and (3) group with LVWT greater than or equal to the sex-specific 90th percentile. A total of 77 participants were excluded because of prevalent congestive heart failure (n=13), prior myocardial infarction (n=27), serum creatinine >2 mg/dL or missing values (n=17), or missing other covariates (n=20). Another 51 individuals with clinically apparent cardiovascular disease were excluded.

Our study sample included 1006 Framingham Offspring participants with available carotid ultrasound measures, and any of the circulating biomarkers, TIMP-1 (n=999), PIIINP (n=915) and/or MMP-9 (n=680).

The Institutional Review Board of Boston University Medical Center approved the study protocol and all participants provided written informed consent.

Plasma MMP-9 and TIMP-1 Measurements

Plasma concentrations of MMP-9, TIMP-1 and/or PIIINP were measured on a subsample of attendees at the sixth examination of the Framingham Offspring (1995–1998), as noted above. Blood samples were drawn from participants who were in a fasting state, and then centrifuged, and the plasma was frozen at −70 °C until assay. Plasma total MMP-9 was measured in duplicate with the use of a 2-site sandwich ELISA assay (Amersham Pharmacia Biotech; assay range of 4 to 128 ng/mL), which measures MMP-9, ProMMP-9, and the ProMMP-9/TIMP-1 complex.(16) Plasma total TIMP-1 was measured similarly with a 2-site sandwich ELISA assay (Amersham Pharmacia Biotech), which measures free TIMP-1 and TIMP-1 complexed with various MMPs.(17) The intra-assay coefficient of variation was <18% for MMP-9 and <5% for TIMP-1 measurements.(16) Plasma PIIINP was measured in duplicate using a radioimmunoassay (Amersham Pharmacia Biotech), and the mean intra-assay coefficient of variation was 6%.

Carotid Ultrasound

Carotid ultrasound was acquired by a trained sonographer, following a standardized protocol. (18) Ultrasound studies were conducted on 3377 of 3532 (96%) of examination cycle 6 participants (1995 –1998). A scan sequence was recorded with a high-resolution linear-array

transducer equipped with a pulsed Doppler spectral analyzer (Model SSA-270A; Toshiba America Medical Systems, Tustin, CA). Scanner settings (four real-time images, carrier frequency, 6.7 MHz; -3 db point) were set at the beginning of the examination and were not altered during the procedure.

Determination of carotid stenosis—Images were analyzed by one operator and over-read by one of the investigators (J.F.P.). Two images were obtained at the level of the distal common carotid artery, 2 at the carotid artery bulb, and 2 at the proximal 2 cm of the internal carotid artery. Internal carotid artery stenosis was graded by the sonographer as 0, 1–24, 25–49% when Doppler derived peak-systolic velocities in the internal carotid artery were <150 cm/s. Hemodynamically significant stenosis (≥50%) was defined by peak-systolic velocities ≥150 cm/s. The degree of stenosis was based on the maximum stenosis in either internal carotid artery. Good intra-reader reproducibility of carotid stenosis assessment (≥25%) in the Framingham Heart Study has been reported elsewhere (Kappa value = 0.69).(19)

Measurements of IMT—IMT was measured at 3 sites of both carotid arteries with all images gated to diastole. The maximal IMT at each site, defined as the mean of the maximal IMT measurements of the near and far walls, correlates well with cardiovascular risk in prior studies.(1) The internal carotid/bulb IMT was defined as the mean of the 4 maximal IMT measurements for the carotid artery bulb and the internal carotid artery on both sides. The common carotid IMT was defined as the mean of the maximal IMT measurements for the right and left common carotid arteries.

Risk factors data

Systolic blood pressures were recorded as the average of two physician measurements. Participants were categorized as current smokers or non smokers based on reported smoking during the year preceding the sixth examination cycle; diabetes mellitus was defined as fasting blood glucose of ≥126mg/dl, a previous diagnosis of diabetes or being on hypoglycemic medication or insulin; prior cardiovascular disease included coronary artery disease, congestive heart failure and peripheral vascular disease.(20)

Statistical Analysis

Multivariable linear and logistic regression analyses were used to determine the associations of matrix biomarkers with IMT and carotid stenosis (≥25%), respectively. Separate analyses were performed for internal carotid IMT and common carotid IMT, both of which were logarithmically-transformed to normalize their distributions. The cut point of 25% for carotid stenosis has been used in previous carotid ultrasound studies in Framingham and other cohorts.(21)

Plasma MMP-9 was modeled as a binary variable (detectable versus undetectable) in all analyses because it was detectable (lower detection limit of 4 ng/mL) in only 20% of individuals. TIMP-1 and PIIINP were both logarithmically-transformed to normalize their skewed distributions. We analyzed age- and sex-specific quartiles of TIMP-1 and PIIINP levels to investigate possible non-linear associations with carotid stenosis and IMT.

All analyses were adjusted for the following covariates: age, sex, stroke risk factors: history of cardiovascular disease, atrial fibrillation, left ventricular mass; current smoking; systolic blood pressure, use of antihypertensive therapy, and diabetes. In order to account for the sampling scheme we additionally adjusted for left ventricular mass.

All analyses were specified a priori and performed using Statistical Analyses System software version 9.1 (SAS Institute, Cary, NC). For these analyses we considered a two-sided p-value <0.05 statistically significant.

Results

Clinical characteristics for the study participants are shown in Table 1. Carotid artery stenosis >25% was observed in 168 participants (17%).

MMP-9 was detectable in 680 participants (20%). The median plasma concentration was 31 ng/dl in men and 34 ng/ml in women (range 20–248 ng/ml).

Relation of biomarkers and carotid ultrasound measures (Table 2)

Detectable MMP-9 concentrations were significantly associated with carotid stenosis (Odds Ratio [OR] 1.71, 95% Confidence interval [CI] 1.05 – 2.80, p=0.032). There was no significant association of MMP-9 with internal or common carotid artery IMT.

The associations of TIMP-1 with carotid stenosis and with carotid IMT were non linear. Higher TIMP-1 (Q4 versus Q1-3) was associated with both carotid stenosis (OR: 1.63, 95% CI 1.07 – 2.48, p=0.022) and with higher internal carotid IMT (β 0.057 ± 0.025 Q4 versus Q1-3, p=0.023), but not with common carotid IMT.

To evaluate further site-specific differences in the associations of TIMP-1 and IMT, we analyzed the effect of higher TIMP-1 (Q4) on IMT, with site of IMT measurement (internal versus common carotid artery) as a factor. There was a significant interaction between higher TIMP-1 and site, even after adjusting for vascular risk factors (p=0.04).

Higher PIIINP (Q4 versus Q1-3) showed a trend towards an association with carotid stenosis (OR 1.45, 95% CI 0.94–2.25, p=0.095) but not with either common or internal carotid IMT.

Discussion

Principal Findings

The present study demonstrates site-specific relations of circulating biomarkers of extracellular matrix remodeling (ECM) and the carotid artery atherosclerosis measures assessed using duplex ultrasound. First, we noted significant associations of higher levels of TIMP-1 with internal carotid IMT, but not with common carotid IMT. The associations with internal carotid IMT remained statistically significant after adjustment for traditional vascular risk factors. Second, higher concentrations of TIMP-1 and detectable MMP-9 were associated with internal carotid artery stenosis, with a trend observed for higher PIIINP. Third, detectable MMP-9 was not associated with internal or common carotid IMT.

Potential mechanisms underlying the observed associations

Tissue inhibitors of metalloproteinases (TIMPs) regulate strictly the activity of MMPs; TIMP-1 in particular is an inhibitor of MMP-9.(22) Both TIMPs and MMPs have been implicated in the process of atherosclerosis.(3,4) The atherosclerotic process affects predominantly vessel bifurcations, such as the carotid artery bifurcation and proximal segment of the internal carotid artery. This finding is likely related to the fact that individual segments of the carotid artery are differentially exposed to turbulent flow, with higher shear forces acting at the internal carotid artery and carotid bifurcation.(23) Adaptive remodeling of the vessel wall in adjusting to altered local mechanical stresses takes place to maintain normal values of tensile stress. This remodeling involves intimal thickening, which may strengthen the arterial wall.(24) Carotid

IMT and carotid stenosis reflect different degrees of severity of atherosclerosis; whereas IMT is an intermediate marker of atherosclerosis and a subclinical marker, stenosis of an artery develops when the vessel in no longer capable of compensating by eccentric remodeling, and intrusion of the atherosclerotic lesion into the vessel lumen occurs, resulting in stenosis.

The findings in our study are supportive of these observations, and consistent with differences in ECM remodeling of different carotid artery segments. Although detectable MMP-9 was not significantly associated with carotid IMT in either the internal or common carotid arteries in this study, higher TIMP-1 showed a significant association. Imbalance of MMPs and TIMPs has been related to the development of atherosclerotic plaques, as shown by pathological studies.(4,25) Upregulation of TIMP-1 has been suggested to counteract over expression of MMPs; however, this may not be sufficient to arrest the process of atherosclerosis, as suggested by prior studies showing that the ratio of MMP-9/TIMP-1 was significantly higher in plaques with plaque disruption,(2) in plaques compared to adjacent carotid regions(25) and lower plasma concentrations of TIMP-1 in patients with unstable plaques compared to those with stable plaques and to healthy volunteers.(2)

In our study both biomarkers, MMP-9 and TIMP-1, were significantly associated with carotid stenosis, supporting the notion that ECM remodeling takes place in different degrees / phases of atherosclerosis. The lack of association of MMP-9 with IMT but the significant association with carotid stenosis suggests that the relation of MMP-9 is stronger with more severe changes of atherosclerosis.

Increased MMP-9 activity,(26) upregulation of the MMP-9 gene and protein(2) have been demonstrated in carotid endarterectomy specimens from patients with severe symptomatic carotid stenosis; in addition increased expression of other MMPs capable of activating MMP-9 such as MMP-3 has been found in carotid atherosclerotic lesions.(9)

MMP-9 has a broad range of substrates among which collagen type III is included. Collagen III is one of the main components of the plaque cap,(27,28) and its turnover may be assessed by measuring the amino-terminal propeptide of type III procollagen (PIIINP). This peptide is an extension of the procollagen type III that is liberated into serum after cleavage during the conversion of type III procollagen to type III collagen. Elevated serum PIIINP is believed to reflect enhanced collagen turnover.(29) The borderline association of higher PIIINP concentrations with carotid stenosis is in agreement with ECM remodeling participating in the process of carotid atherosclerosis.

Strengths and Limitations

The strengths of our study include the community-based sample, use of reproducible quantitative carotid ultrasound techniques, interpretation of biomarkers and ultrasound imaging data independent of each other and blinded to clinical data.

One of the limitations of our study is the sampling design for the ECM biomarkers based on the distribution of left ventricular remodeling phenotypes. However, we adjusted for measures of left ventricular mass in our analyses and the association between elevated biomarkers and carotid ultrasound markers was maintained.

Although blood concentration of the biomarkers evaluated may be affected by systemic processes, all samples were collected following a standard protocol, under resting conditions; participants were excluded if they had congestive heart failure, a history of myocardial infarction and none of the participants were known to have cancer within the 6 months preceding the 6^{th} cycle examination.(17) In addition, evaluation of the TIMP/MMP pathway was limited in this study, and future studies could include measurements of enzyme activity

and other MMPs. Lastly, the Framingham Heart Study participants are of white of European descent; further studies are required to confirm our findings in other ethnic and racial groups and expand the results to larger populations.

Conclusions

Higher circulating concentrations of ECM turnover markers, TIMP-1, MMP-9 and PIIINP, are associated with measures of carotid artery atherosclerosis after adjusting for standard risk factors in a community-based sample of middle-aged adults, free of clinical stroke and dementia. Our findings are consistent with site-specific carotid artery extracellular matrix remodeling and support a pathophysiological role for the MMP/TIMP pathway in atherosclerosis of the carotid artery.

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References

1. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med 1999;340:14–22. [PubMed: 9878640]
2. Sapienza P, di Marzo L, Borrelli V, et al. Metalloproteinases and their inhibitors are markers of plaque instability. Surgery 2005;137:355–363. [PubMed: 15746792]
3. Johnson JL, Jackson CL, Angelini GD, George SJ. Activation of matrix-degrading metalloproteinases by mast cell proteases in atherosclerotic plaques. Arterioscler Thromb Vasc Biol 1998;18:1707–1715. [PubMed: 9812908]
4. Loftus IM, Goodall S, Crowther M, et al. Increased MMP-9 activity in acute carotid plaques: therapeutic avenues to prevent stroke. Ann N Y Acad Sci 1999;878:551–554. [PubMed: 10415770]
5. Ghilardi G, Biondi ML, DeMonti M, Turri O, Guagnellini E, Scorza R. Matrix metalloproteinase-1 and matrix metalloproteinase-3 gene promoter polymorphisms are associated with carotid artery stenosis. Stroke 2002;33:2408–2412. [PubMed: 12364729]
6. Alvarez B, Ruiz C, Chacon P, Alvarez-Sabin J, Matas M. Serum values of metalloproteinase-2 and metalloproteinase-9 as related to unstable plaque and inflammatory cells in patients with greater than 70% carotid artery stenosis. J Vasc Surg 2004;40:469–475. [PubMed: 15337875]
7. Formato M, Farina M, Spirito R, et al. Evidence for a proinflammatory and proteolytic environment in plaques from endarterectomy segments of human carotid arteries. Arterioscler Thromb Vasc Biol 2004;24:129–135. [PubMed: 14592849]
8. Cunningham LA, Wetzel M, Rosenberg GA. Multiple roles for MMPs and TIMPs in cerebral ischemia. Glia 2005;50:329–339. [PubMed: 15846802]
9. Galis ZS, Sukhova GK, Lark MW, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J Clin Invest 1994;94:2493–2503. [PubMed: 7989608]
10. Heiss G, Sharrett AR, Barnes R, Chambless LE, Szklo M, Alzola C. Carotid atherosclerosis measured by B-mode ultrasound in populations: associations with cardiovascular risk factors in the ARIC study. Am J Epidemiol 1991;134:250–256. [PubMed: 1877584]
11. Mackinnon AD, Jerrard-Dunne P, Sitzer M, Buehler A, von Kegler S, Markus HS. Rates and determinants of site-specific progression of carotid artery intima-media thickness: the carotid atherosclerosis progression study. Stroke 2004;35:2150–2154. [PubMed: 15243147]
12. Wagenknecht LE, Zaccaro D, Espeland MA, Karter AJ, O'Leary DH, Haffner SM. Diabetes and progression of carotid atherosclerosis: the insulin resistance atherosclerosis study. Arterioscler Thromb Vasc Biol 2003;23:1035–1041. [PubMed: 12702517]

13. Lynch J, Kaplan GA, Salonen R, Salonen JT. Socioeconomic status and progression of carotid atherosclerosis. Prospective evidence from the Kuopio Ischemic Heart Disease Risk Factor Study. Arterioscler Thromb Vasc Biol 1997;17:513–519. [PubMed: 9102170]
14. Yanez ND 3rd, Kronmal RA, Shemanski LR, Psaty BM. A regression model for longitudinal change in the presence of measurement error. Ann Epidemiol 2002;12:34–38. [PubMed: 11750238]
15. Rubba P, Panico S, Bond MG, et al. Site-specific atherosclerotic plaques in the carotid arteries of middle-aged women from southern Italy: associations with traditional risk factors and oxidation markers. Stroke 2001;32:1953–1959. [PubMed: 11546880]
16. Sundstrom J, Evans JC, Benjamin EJ, et al. Relations of plasma matrix metalloproteinase-9 to clinical cardiovascular risk factors and echocardiographic left ventricular measures: the Framingham Heart Study. Circulation 2004;109:2850–2856. [PubMed: 15173025]
17. Sundstrom J, Evans JC, Benjamin EJ, et al. Relations of plasma total TIMP-1 levels to cardiovascular risk factors and echocardiographic measures: the Framingham heart study. Eur Heart J 2004;25:1509–1516. [PubMed: 15342170]
18. Polak JF, O'Leary DH, Kronmal RA, et al. Sonographic evaluation of carotid artery atherosclerosis in the elderly: relationship of disease severity to stroke and transient ischemic attack. Radiology 1993;188:363–370. [PubMed: 8327679]
19. Elosua R, Ordovas JM, Cupples LA, et al. Association of APOE genotype with carotid atherosclerosis in men and women: the Framingham Heart Study. J Lipid Res 2004;45:1868–1875. [PubMed: 15258198]
20. Wolf PA, D'Agostino RB, Belanger AJ, Kannel WB. Probability of stroke: a risk profile from the Framingham Study. Stroke 1991;22:312–318. [PubMed: 2003301]
21. Wilson PW, Hoeg JM, D'Agostino RB, et al. Cumulative effects of high cholesterol levels, high blood pressure, and cigarette smoking on carotid stenosis. N Engl J Med 1997;337:516–522. [PubMed: 9262494]
22. Matrisian LM. Metalloproteinases and their inhibitors in matrix remodeling. Trends Genet 1990;6:121–125. [PubMed: 2132731]
23. Motomiya M, Karino T. Flow patterns in the human carotid artery bifurcation. Stroke 1984;15:50–56. [PubMed: 6695430]
24. Stary HC, Blankenhorn DH, Chandler AB, et al. A definition of the intima of human arteries and of its atherosclerosis-prone regions. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Arterioscler Thromb 1992;12:120–134. [PubMed: 1731855]
25. Higashikata T, Yamagishi M, Higashi T, et al. Altered expression balance of matrix metalloproteinases and their inhibitors in human carotid plaque disruption: results of quantitative tissue analysis using real-time RT-PCR method. Atherosclerosis 2006;185:165–172. [PubMed: 16039658]
26. Loftus IM, Naylor AR, Goodall S, et al. Increased matrix metalloproteinase-9 activity in unstable carotid plaques. A potential role in acute plaque disruption. Stroke 2000;31:40–47. [PubMed: 10625713]
27. Katsuda S, Okada Y, Minamoto T, Oda Y, Matsui Y, Nakanishi I. Collagens in human atherosclerosis. Immunohistochemical analysis using collagen type-specific antibodies. Arterioscler Thromb 1992;12:494–502. [PubMed: 1373075]
28. Burleigh MC, Briggs AD, Lendon CL, Davies MJ, Born GV, Richardson PD. Collagen types I and III, collagen content, GAGs and mechanical strength of human atherosclerotic plaque caps: span-wise variations. Atherosclerosis 1992;96:71–81. [PubMed: 1418104]
29. Bode MK, Mosorin M, Satta J, Risteli L, Juvonen T, Risteli J. Complete processing of type III collagen in atherosclerotic plaques. Arterioscler Thromb Vasc Biol 1999;19:1506–1511. [PubMed: 10364082]

TABLE 1
Characteristics of study sample

	Men (n=439)	Women (n=567)
Clinical Characteristics		
Age, yrs, mean [SD]	58 [10]	58 [10]
Systolic blood pressure, mm Hg, mean [SD]	128 [18]	125 [21]
Anti-hypertensive medication, %	27	23
Current smokers, %	17	12
Diabetes mellitus, %	12	6
Prevalent cardiovascular disease, %	13	7
Atrial fibrillation, %	5.9	1.6
Electrocardiographic LVH, %	2.5	0.0
Biochemical Characteristics		
MMP-9 detectable [n=680]	22%	19%
TIMP-1 median (range) [n=999], ng/ml	802 (412–3146)	754 ([430–1760)
PIIINP median (range) [n=915], ng/ml	3.38 (0.54–36.52)	3.03 (0.02–28.42)
Carotid Ultrasound Measures		
Common Carotid IMT median (range) mm [n=1005]	0.70 (0.46–1.83)	0.67 (0.46–1.45)
Internal Carotid IMT median (range) mm [n=1001]	0.88 (0.44–5.02)	0.72 (0.40–3.06)
Carotid Stenosis ≥25% [n=1006]	23%	12%

TABLE 2

Relation of biomarkers and carotid ultrasound measures

	Carotid Stenosis ≥25%		Log (CCA IMT)		Log (ICA IMT)	
TIMP-1	**OR (95% CI)**	**p**	**β (95% CI)**	**p**	**β (95% CI)**	**p**
Log (TIMP)	**1.28 [1.05–1.56]**	**0.015**	0.006 ± 0.006	NS	**0.029 ±0.012**	**0.016**
Q1	Referent	NS*	Referent	NS*	Referent	NS*
Q2	0.74 [0.42–1.34]		−0.026 ± 0.014		−0.018 ± 0.030	
Q3	0.82 [0.46–1.45]		−0.001 ± 0.014		−0.016 ± 0.030	
Q4	1.38 [0.81–2.37]		0.002 ±0.015		0.045 ± 0.031	
Q4 versus Q1-Q2-Q3	**1.63 [1.07–2.48]**	**0.022**	0.008 ± .0012	NS	**0.057 ± 0.025**	**0.023**
PIIINP						
Log (PIIINP)	1.16 [0.93–1.43]	NS	0.003 ± 0.005	NS	0.005 ± 0.011	NS
Q1	Referent	0.063*	Referent	NS*	Referent	0.064*
Q2	1.09 [0.60–1.98]		−0.020 ± 0.015		−0.012 ± 0.031	
Q3	1.24 [0.69–2.20]		0.009 ± 0.015		0.073 ± 0.031	
Q4	1.61 [0.92–2.83]		0.000 ± 0.015		0.025 ± 0.031	
Q4 versus Q1-Q2-Q3	1.45 [0.94–2.25]	NS	0.004 ± 0.012	NS	0.005 ± 0.026	NS
MMP-9						
Detectable	**1.71 [1.05–2.80]**	**0.032**	−0.012 ± 0.016	NS	0.046 ± 0.034	NS
Not Detectable	Referent		Referent		Referent	

CCA=common carotid artery; ICA=internal carotid artery.

*P value for trend.