Published in final edited form as:
Atherosclerosis. 2010 May ; 210(1): 188–193. doi:10.1016/j.atherosclerosis.2009.12.006.

MMP2 Genetic Variation Is Associated With Measures of Fibrous Cap Thickness: The Atherosclerosis Risk in Communities Carotid MRI Study

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Abstract

Objective—Genetic variation in matrix metalloproteinase (MMP) promoter regions alters the transcriptional activity of MMPs and has been consistently associated with CHD, presumably through plaque degradation and remodeling. We examined the association of MMP promoter variation with multiple plaque characteristics measured by gadolinium-enhanced MRI among 1,700 participants in the Atherosclerosis Risk in Communities (ARIC) Carotid MRI Study.

Methods—For the analyses presented here, 1,700 participants of the biracial ARIC Carotid MRI Study (~1,000 participants with thick carotid artery walls and ~700 randomly sampled participants) were evaluated for associations of MMP genetic variation with multiple plaque characteristics,

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Conflicts of Interest: None

including carotid artery wall thickness, lipid core and fibrous cap measures. MRI studies were performed on a 1.5T scanner equipped with a bilateral 4-element phased array carotid coil.

Results—Fifty-one percent of the participants were female, 77% white, 23% African American, and the mean age was 70 years. MMP2 C-1306T variant genotypes (CT+TT) were significantly associated with higher cap thickness measures, but not with wall thickness or lipid core measures. Individuals with the CC genotype had approximately 0.1 mm thinner cap thickness compared to those carrying a T allele (p=0.02).

Conclusion—Genetic variation within the MMP2 promoter region was associated with cap thickness and therefore may influence the role of MMP2 in plaque vulnerability.

Keywords

Atherosclerosis; Carotid MRI; Vulnerable Plaque; MMP; Genetics

Introduction

Matrix metalloproteinases (MMPs) are a family of enzymes present and enzymatically active in atherosclerotic plaques (1-3). MMP expression is primarily regulated at the transcription level, with single nucleotide polymorphisms (SNPs) in the promoter regions of MMPs shown to alter transcriptional activity and contribute to cardiovascular disease susceptibility (1,4-6). MMPs can degrade a range of extracellular matrix proteins and are implicated in connective tissue degradation and remodeling associated with the development of atherosclerotic lesions.

Both observational and functional studies have investigated the role of MMP genetic variation with regards to gene expression, disease susceptibility and vascular remodeling (1,5-8). Studies indicate that MMP genetic variation may contribute to individual differences in complex disease susceptibility through effects on the balance between the synthesis and degradation of extracellular matrix proteins (1,5-8). In previous studies, MMP2, MMP3 and MMP12 gene promoter variations have been associated with coronary heart disease (CHD) (2,6-18). The mechanism of the association between MMP gene variation and CHD is presumed to be the result of the effects of the MMP activity on fibrous cap thickness and the genesis of vulnerable plaques (18).

In this study, we used gadolinium-enhanced carotid MRI to measure atherosclerotic plaque characteristics among 1,700 participants in the Atherosclerosis Risk in Communities (ARIC) study and to examine the association between targeted MMP gene variation and plaque characteristics, with specific *a priori* hypotheses related to fibrous cap thickness. The genes and SNPs that were selected were those that have previously been associated with CHD in multiple population-based or case-control studies.

Methods

Study Design and Study Participants

The ARIC Carotid MRI Study was conducted between 2004 and 2005. The participants were selected from the ARIC study cohort based on results of the last ultrasound examinations (Visits 3 and 4, 1993-1998). The ARIC study is a prospective investigation of atherosclerosis and its clinical sequelae involving 15,792 African American and white men and women aged 45 to 64 years at recruitment (1987-1989). Participants underwent a baseline and up to three follow-up visits through 1998. A detailed description of the ARIC study design and methods has been published elsewhere (19). Two groups were recruited for the ARIC Carotid MRI Study: ~1,200 participants with thick carotid artery walls based on ultrasound exams from visits 3 and 4 (>85th percentile intima-media thickness (IMT); “high IMT group”) and ~800 participants

randomly sampled from the remainder of the cohort (<85th percentile IMT; "not-high group"). The carotid artery IMT cutpoint was determined for each field center based on the IMT distribution specific to that site. The participants were screened for contraindications to MRI or to contrast media. In addition, selected participants also received in-person interviews and a physical examination. The study was approved by local Institutional Review Boards. For the current analyses, participants were excluded if they prohibited use of their DNA for research purposes or had missing information for all measurement variables. Following exclusions, a total of 1,701 participants were available for analysis.

MRI Protocol

MRI studies were performed on a 1.5T scanner (GE Medical Systems, Milwaukee, WI at three 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 MR angiogram was acquired through the extracranial carotid bifurcations and this was used to identify the carotid artery with the thicker wall or plaque. Each participant received an intravenous injection of gadodiamide (Omniscan, GE Amersham), 0.1 mmol/kg body weight, using a power injector. Sixteen axial T1-weighted, fat-suppressed BBMRI slices (thickness, 2mm; acquired in-plane resolution, 0.51×0.58mm^2; 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 five minutes after the intravenous injection of gadodiamide (Omniscan, GE Amersham), 0.1 mmol/kg body weight, with a power injector.

Image Analysis

MRI images were analyzed by seven readers, blinded to the clinical and laboratory characteristics of the study population, using semiautomated software (VesselMASS, The Netherlands). 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 slices 4 through 11 were analyzed. If these 8 slices did not contain the thickest part of the plaque, then the reader continued contouring in the direction of the thickest wall (i.e. slices 1 through 11 or slices 4 through 16).

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 (20,21) and contours were drawn to delineate the lumen, lipid core, and outer wall. The fibrous cap contour was automatically generated based on the lipid core and lumen contours. Using semi-automated software, the vessel walls were divided into 12 radial segments. Mean, minimum, and maximum thickness values were generated for each segment. The fibrous cap was divided into radial segments at 15° increments, and mean, minimum, and maximum thickness measurements were generated for each segment. Area measurements also were generated for the lipid core contours. Volumetric data were computed by integrating area measurements over all slices.

Examination and Laboratory Measures

All measures utilized in these analyses were from the 2004-2005 MRI exam. Seated blood pressure was measured three times with a random-zero sphygmomanometer and the last two measurements were averaged. Hypertension was defined as systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg or current use of antihypertensive medications. Questionnaires and in-person interviews were used to assess use of antihypertensive medications. Diabetes was defined by a fasting glucose level ≥ 126 mg/dL, a non-fasting glucose level ≥ 200 mg/dL, and/or history of or treatment for diabetes. Cigarette-smoking status was analyzed by comparing current smokers to individuals who had formerly or never smoked.

Body mass index (BMI, kg/m^2) was calculated from height and weight measurements. Plasma total cholesterol was measured by an enzymatic method (22). High-density lipoprotein (HDL) cholesterol was measured after dextran-magnesium precipitation of non-HDL lipoproteins (23).

Genotype Determination

Known functional promoter or missense mutations in the matrix metalloproteinase (MMP) gene family or SNPs previously reported to be associated with cardiovascular conditions were selected for investigation. The polymorphisms included: MMP2 G-1575A (rs243866), MMP2 C-1306T (rs243865), MMP2 T-790G (rs243864), MMP3 5A/6A (rs35068180), MMP3 K45E (rs679620), MMP7 A-181G (rs11568818), MMP7 C-153T (rs11568819), MMP9 R279Q (rs17576), MMP12 A-82G (rs2276109) MMP13 A-77G (rs2252070). Genotyping was carried out using the SNPlex or TaqMan Assays (Applied Biosystems, Inc, Foster City, CA). Primers and probes are available from the authors upon request.

Statistical Analysis

All analyses were based on methods appropriate for a stratified random sample. In particular, all analyses were weighted by the inverse of the sampling fractions in the eight sampling strata (4 field centers × 2 IMT groups). The sampling fractions were based on those persons actually screened for participation. Those who actually participated (non-refusing eligibles) were analyzed as a sub-population in calculating variances and confidence intervals of estimators. Analyses were conducted utilizing SAS version 9.1 for descriptive statistics (PROC SURVEYMEANS, PROC SURVEYFREQ) and SUDAAN for linear and logistic regression. 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 REGRESS for continuous variables and LOGISTIC for dichotomous variables, with predicted values calculated as sample means of the adjusting variables.

Genotype frequencies were calculated as weighted frequencies using SUDAAN CROSSTAB, and allele frequencies were calculated from genotype frequencies as P(AA) + P(AB)/2, where P(AA) and P(AB) are the weighted frequencies of AA and AB, respectively. The test for Hardy-Weinberg equilibrium used the weighted genotype frequencies and their covariance matrix, applying the delta method to obtain the test statistic. Variant alleles were identified as the low frequency allele in whites, and homozygous wildtype (non-variant) genotypes were designated as the referent group in the statistical analyses.

Results

Descriptive statistics of the ARIC Carotid MRI Study participants are presented in Table 1 stratified by race and gender. African Americans had higher mean LDL cholesterol and a higher occurrence of diabetes and hypertension compared to whites. Whites had a higher percentage of former smokers and statin users compared to African Americans. Carotid artery wall thickness, lipid core and cap measures are presented in Table 2 stratified by race and gender. Statistically significant race-gender differences were observed for all carotid artery wall thickness and lipid core measures. Men, especially white men, had greater wall thickness than the women. White men also had a larger percent of plaques with a lipid core and a greater lipid core volume, when present, compared to the other groups. No significant race-gender differences were observed for cap measures.

All MMP genotype distributions were in accordance with Hardy-Weinberg equilibrium expectations. Statistically significant associations were observed between the three MMP2 SNPs (rs243864, rs243865, rs243866) and all cap measures. No significant associations were observed for the other MMP SNPs studied. The allele frequencies for each of the three MMP2 SNPs were similar within each racial group, but allele frequencies were disparate between whites and African Americans. Allele frequencies for each MMP2 SNP were as follows for whites and African Americans, respectively: rs243864 f(G)=0.23 and 0.06, rs243865 f(T)=0.23 and 0.07, rs243866 f(A)=0.23 and 0.06. Further examination of the linkage disequilibrium (LD) pattern of the MMP2 gene showed these three SNPs to be in high LD with one another (in a single LD block; r^2=1.0 for all three SNPs). Therefore, we present here the results for the single MMP2 SNP, rs243865, which was designated as the tagSNP for this MMP2 linkage block. Results for the additional MMP2 SNPs investigated (rs243864 and rs243866) are provided in supplemental tables available on-line.

Carotid artery wall thickness, lipid core and cap measures for the ARIC Carotid MRI participants by MMP2 rs243865 genotype are presented in Table 3. MMP2 variant genotypes were not significantly associated with measures of carotid artery wall thickness or lipid core measures, but were consistently associated with significantly higher cap thickness measures. This association was present and statistically significant over a wide variety of adjustments that were explored (data not shown). Individuals with the CC genotype had approximately 0.1 mm thinner cap thickness compared to those carrying a T allele. This effect was also evident when the analysis was restricted to the sample of white participants. The mean cap thickness in whites with the CC genotype was 0.637 compared to 0.746 for those carrying a T allele (p=0.02). There were insufficient numbers of African Americans with cap measurements to support race-specific analyses in that group.

Discussion

An atheromatous plaque is a complex lesion composed of distinct morphological features, including a fibrous cap, a shoulder area and a lipid core. Histological studies have demonstrated that specific characteristics of the plaque structure influence the risk of plaque rupture (24). A plaque with a thin fibrous cap and a large lipid core is more vulnerable to rupture, whereas a plaque with a thick fibrous cap and a small lipid core is considered to be stable (25-27). It has been postulated that thin cap fibroatheroma are the precursor lesion of plaque rupture (25). In concurrence with histological evidence, noninvasive high-resolution MRI has shown a significant association between the *in vivo* state of the fibrous cap and recent history of stroke (28), as well as the importance of fibrous cap status in the development of future ischemic complications (29). Since plaque rupture and formation may result in thrombosis and an acute cardiovascular disease event, it is important to identify factors that modify the plaque structure, especially those that lead to instability by causing the formation of a thin cap and large lipid core.

The extracellular matrix (ECM) of the fibrous cap, comprised of multiple macromolecules including collagen and smooth muscle cells, provides the structural integrity of a plaque and contributes to plaque formation and progression (30). Although the mechanisms underlying plaque rupture are incompletely known, MMPs play a central role in the degradation of ECM components and therefore may contribute to the destabilization and thinning of the fibrous cap and subsequent plaque rupture (5,8,30,31). MMP degradation of the ECM can be influenced by a number of regulatory mechanisms, the most notable being transcriptional regulation (6). MMP transcript levels have been shown to be higher in thin cap plaques compared to plaques with a thick fibrous cap, as well as MMP transcript levels being increased in ruptured plaques compared with lesions without cap disruption (32).

Experimental studies have reported that MMP2 and MMP9, both gelatinase MMPs, are overexpressed and enzymatically active within vulnerable sites of human atheroma (4,18,31, 33). MMP2 and MMP9 are considered to be involved in plaque stability/instability due to their role in the cleaving of matrix proteins (e.g. type IV collagen) and non-matrix substrates (e.g. CD-44, cadherins) which promote migration, proliferation and viability of vascular smooth muscle cells, all of which are processes expected to favor plaque-cap stability (34-36). A recent study by Kuge and colleagues showed MMP2 immunohistochemical staining to be significantly and positively correlated with morphological vulnerability of atheromatous lesions in hypercholesterolemic rabbits with pathological characteristics relevant to human atherosclerosis (34). The findings from Kuge and colleagues, as well as previous studies, support the hypothesis that MMP2 activity plays a pivotal role in the destabilization of atherosclerotic plaques (4,18,31,33,34). Immunohistochemical staining for MMP2 has also been observed to be more prevalent in plaques with expansive remodeling, which may predispose to plaque rupture (37-40).

The MMP2 C-1306T variant is a functional SNP that abolishes a Sp1 binding site within the promoter region of the MMP2 gene and thus results in lower promoter activity (11). Genotypes carrying the variant −1306T allele have been shown to result in lower levels of MMP2 mRNA, and therefore lower gene expression and lower MMP2 levels (41). These observations support our hypothesis that persons carrying the variant −1306T allele have decreased MMP2 expression/levels and thus have thicker caps compared to persons with the −1306CC genotype. Presence of the −1306T allele would thus favor plaque-cap stability. For the current study, MMP2 levels were available on a subset of the population. The MMP2 C-1306T variant was associated with MMP2 levels, with lower levels observed in persons carrying the −1306T variant allele compared to persons with the −1306CC genotype (CC = 304.0 ± 1.1 ng/ml, CT +TT = 301.1 ± 1.6 ng/ml, P=0.1).

We observed that compared to individuals carrying the T allele, individuals with the CC genotype had approximately 0.1 mm thinner caps, a difference of approximately 15% of the mean cap thickness. The lower mean cap thickness for those individuals with the CC genotype (0.64 mm) will include a range of thicknesses that encompass more values below the pathologically-determined threshold for rupture (165 microns) than those individuals carrying the T allele (42). This suggests a higher susceptibility to rupture with thrombus formation for those with the CC genotype, potentially putting these individuals at a higher risk for stroke. Limitations to the current study include replication, the technology of the MRI and plaque characterization, and correction for multiple testing. First of all, we were unable to replicate our findings due the uniqueness of the MRI phenotypes characterized in the ARIC Carotid MRI study. Second, the resolution constraints of the MRI scanner limited our ability to detect features within the smallest plaques, so only the largest plaques found within the thickest arterial walls were studied to limit the impact of these constraints. By truncating the distribution of lipid core volume and cap thickness, we have thereby restricted the range of values and possibly the association with MMP genetic variation. Although the submillimeter fibrous cap thickness measurements were limited by our scan resolution, we observed moderate reliability for these measurements as reported by Wasserman et al. (43), perhaps due to the reader's ability to visually interpolate the cap boundaries with approximate accuracy. With regards to multiple testing, the fact that statistically significant associations were observed for all three MMP2 SNPs (rs243864, rs243865, rs243866) and all cap measures provides evidence that these associations are not due to chance alone. Despite these limitations, the strengths of the present study include its being a well-characterized sample of a population-based cohort utilizing a standardized MRI protocol with central reading and reliability data and a novel phenotype.

MMPs have established functions that might increase or decrease plaque growth and stability through a variety of mechanisms (36). Previous studies of genetic variants in MMP gene

promoter regions have revealed associations with atherosclerotic plaque progression and stability (36). Although replication of the current study is warranted in other populations, results from the current study show that variation within the promoter region of the MMP2 gene is associated with cap thickness and therefore may influence the role of MMP2 in plaque vulnerability.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The Atherosclerosis Risk in Communities Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts N01-HC-55015, N01-HC-55016, N01-HC-55018, N01-HC-55019, N01-HC-55020, N01-HC-55021, and N01-HC-55022. The authors thank the staff and participants of the ARIC study for their important contributions.

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Table 1

Descriptive Statistics of ARIC Carotid MRI Study Participants*

Characteristics	African-American Women (N=230)	African-American Men (N=159)	White Women (N=640)	White Men (N=672)
Age (years)	69.2 (0.4)	69.0 (0.5)	70.3 (0.3)	71.0 (0.3)
LDL Cholesterol (mg/dl)	127.1 (3.2)	119.1 (2.9)	118.8 (1.6)	105.5 (1.8)
HDL Cholesterol (mg/dl)	54.9 (1.2)	45.6 (1.0)	55.3 (0.8)	43.7 (0.5)
Systolic Blood Pressure (mmHg)	127.3 (1.6)	127.4 (2.1)	126.5 (0.9)	123.3 (0.9)
Diastolic Blood Pressure (mmHg)	66.9 (0.8)	71.4 (1.1)	65.8 (0.5)	67.2 (0.5)
BMI (kg/m^2)	30.5 (0.4)	28.4 (0.4)	28.0 (0.3)	28.1 (0.2)
Current Smoker	8.2%	13.5%	7.5%	6.8%
Former Smoker	28.5%	42.6%	33.1%	58.6%
Hypertensive	79.2%	67.6%	61.8%	57.4%
Hypertensive medication use	78.2%	62.0%	62.2%	57.6%
Diabetes	31.1%	39.9%	17.7%	22.1%
Diabetes Medication use	19.6%	28.8%	10.8%	13.7%
Statin use	23.4%	26.4%	31.9%	41.0%

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

* Carotid MRI participants defined as those with complete quality MRI scan data and not restricted to those with genotype data

Table 2

Carotid Artery Wall Thickness, Lipid Core and Cap Measures for ARIC Carotid MRI Participants

	African American				White				P*
	N	Women	N	Men	N	Women	N	Men	
Carotid Artery Wall Thickness Measures									
Total Wall Volume (ml)	230	0.35 (0.01)	159	0.43 (0.01)	640	0.38 (0.01)	671	0.49 (0.01)	<0.0001
Max Wall Thickness (mm, segment)	230	1.79 (0.08)	159	1.99 (0.09)	640	1.98 (0.04)	671	2.51 (0.07)	<0.0001
Lipid Core Measures †									
Total Lipid Core Volume (ml)	230	0.01 (0.002)	159	0.01 (0.003)	640	0.01 (0.002)	672	0.03 (0.003)	<0.0001
Mean Lipid Core Area (cm^2)	230	0.01 (0.002)	159	0.01 (0.003)	640	0.01 (0.002)	672	0.03 (0.003)	<0.0001
Percent with Lipid Core		19.1%		17.6%		18.8%		29.6%	0.0003
Lipid Core Measures ‡									
Total Lipid Core Volume (ml)	45	0.05 (0.008)	36	0.06 (0.01)	156	0.06 (0.007)	244	0.09 (0.009)	0.004
Mean Lipid Core Area (cm^2)	45	0.06 (0.007)	36	0.07 (0.01)	156	0.07 (0.005)	244	0.09 (0.008)	0.01
Cap Measures									
Mean Min Cap Thickness (mm, 2 adjacent)	44	0.45 (0.04)	35	0.51 (0.03)	156	0.47 (0.02)	241	0.50 (0.02)	0.6
Mean Cap Thickness (mm, 2 adjacent)	44	0.65 (0.05)	35	0.70 (0.05)	156	0.65 (0.03)	241	0.71 (0.03)	0.5

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

*P-value for test of differences in means and percentages;

†Participants with a missing value for lipid core measures had their values re-set to zero;

‡Includes only those participants with presence of a lipid core in two adjacent slices

Table 3

Carotid Artery Wall Thickness, Lipid Core and Cap Measures for ARIC Carotid MRI Participants by MMP2 rs243865 Genotype

	N	CC	N	CT + TT	P*
Carotid Artery Wall Thickness Measures					
Total Wall Volume (ml)	1031	0.417	554	0.419	0.8
Max Wall Thickness (mm, segment)	1031	2.161	554	2.135	0.7
Lipid Core Measures †					
Total Lipid Core Volume (ml)	1032	0.018	554	0.016	0.6
Mean Lipid Core Area (cm^2)	1032	0.021	554	0.018	0.3
Percent with Lipid Core		22.7%		21.9%	0.8
Lipid Core Measures ‡					
Total Lipid Core Volume (ml)	285	0.073	164	0.068	0.7
Mean Lipid Core Area (cm^2)	285	0.081	164	0.073	0.4
Cap Measures					
Mean Min Cap Thickness (mm, 2 adjacent)	280	0.454	164	0.540	0.01
Mean Cap Thickness (mm, 2 adjacent)	280	0.641	164	0.748	0.02

*P-value for test of differences in means and percentages (all measures adjusted for age, race and sex);

†Participants with a missing value for lipid core measures were coded as zero;

‡Includes only those participants with presence of a lipid core in two adjacent slices