Matrix Gla Protein Gene Polymorphism Is Associated With Increased Coronary Artery Calcification Progression


Objective—Matrix gla protein (MGP) inhibits arterial and cartilaginous calcification. A threonine to alanine (Thr83Ala) polymorphism (codon 83) in MGP is associated with myocardial infarction and femoral artery calcification. We examined the association of the MGP Thr83Ala polymorphism with quantity and progression of coronary artery calcification (CAC), a noninvasive measure of subclinical coronary atherosclerosis.

Methods and Results—In 605 participants of the Epidemiology of Coronary Artery Calcification Study, generalized linear mixed models were fit to determine the association of MGP Thr83Ala with CAC quantity and progression. There was a significant additive relation between MGP Thr83Ala and CAC progression (P=0.001). In the fully adjusted model, every 1 Ala83 allele increase was associated with an estimated 1.9% (95% confidence interval, 0.7%–3.0%) per year since baseline larger increase in CAC quantity. A proxy single nucleotide polymorphism for MGP Thr83Ala (rs6488724) was similarly associated with CAC progression in an independent cohort from the Genetic Epidemiology Network of Arteriopathy (GENOA) study.

Conclusion—Increased risk of myocardial infarction associated with MGP ThrAla83 genotype observed elsewhere may be related to faster progression of subclinical coronary atherosclerosis. MGP genotype could be a potential candidate for identifying individuals at increased risk of atherosclerotic disease who would benefit from aggressive primary prevention strategies. (Arterioscler Thromb Vasc Biol. 2013;33:645-651.)

Key Words: atherosclerosis ■ calcium ■ genetics ■ imaging ■ population

Matrix gla protein (MGP) inhibits vessel and cartilage calcification and is expressed in calcified plaques. MGP knockout mice experience early death as a result of an arterial calcification leading to blood vessel rupture.1 In humans, Keutel syndrome (MIM 245150),2 which results from nonsense mutations in MGP (12p13.1–p12.3), presents with abnormal cartilage calcification.3

Coronary artery calcification (CAC), a measure of coronary atherosclerosis presence and extent, is measured noninvasively with computed tomography (CT). Serial CT measures can be used to track CAC progression with time.4 CAC progression is associated with future coronary heart disease (CHD) events, including myocardial infarction (MI).5,6 CAC quantity7 and progression8 are heritable, although the specific genes involved are largely unknown. Further, the genes for CAC quantity and progression do not completely overlap (ie, incomplete pleiotropy),9 suggesting the need to examine genes involved in both processes.

In some studies,9 although not all,10,11 serum levels of MGP are seen to be associated with CAC. MGP promoter region polymorphisms are associated with variability in serum MGP levels.12 A single nucleotide polymorphism (SNP) in the promoter region of MGP (T-138C substitution) was weakly, but not statistically significantly, associated with CAC presence among black and white participants of the Coronary Artery Risk Development In Young Adults (CARDIA) study.13 In older men taking part in a trial of vitamin K supplementation, MGP polymorphisms were associated with CAC presence and quantity.10 In a recent genome-wide association study of CAC quantity, however, there was no evidence of a common genetic variant in the region of MGP having an association with CAC quantity.14

A Threonine (Thr) to Alanine (Ala) substitution at codon 83 (Thr83Ala; rs4236) of MGP changes a polar amino acid to a nonpolar amino acid in 1 of the 5 Gla-binding domains of MGP.15 The Thr83Ala missense mutation may alter the proteolytic processing of MGP or decrease MGP’s ability to bind to calcium, resulting in calcium depositing in the arterial wall.16,17 Ala83 carriers have increased risk of MI and femoral calcification as measured with B-mode ultrasonography.15

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The Thr83Ala polymorphism is also associated with chronic kidney disease\(^{18}\) and kidney stones\(^{9}\); both chronic kidney disease and kidney stone disease are associated with subclinical atherosclerosis and increased risk of MI.\(^{20-23}\)

To our knowledge, no studies have reported examination of the association between the MGP Thr83Ala substitution and quantity and progression of CAC. Here, we examine these associations among a group of asymptomatic, community-based research participants.

**Patients and Methods**

**Study Participants**

The Rochester Family Heart Study (RFHS), a community-based study of 3974 individuals, 5 to 90 years old, was conducted between 1984 and 1991.\(^{24,25}\) The Epidemiology of Coronary Artery Calcification (ECAC) study included 1736 participants seen between 1991 and 1998. Among the 1736 ECAC study participants, 1032 were identified from the RFHS. The ECAC study participants lived in the vicinity of Rochester, MN, were ≥20 years of age at the time of recruitment, were not pregnant or lactating, and never had coronary or noncoronary heart surgery.\(^{26,27}\) A total of 1155 ECAC participants, including 622 of the original 1032 FHRS participants, had a follow-up examination between December 2000 and February 2005. In general, participants were invited to return for a follow-up examination on the basis of age (older age first) and longer time since baseline examination. The study protocols were approved by the Mayo Clinic and University of Michigan institutional review boards, and participants gave written informed consent.

As part of the RFHS, 665 white participants who also participated in the ECAC study were genotyped for MGP, irrespective of risk factor or disease status. Twelve individuals reporting a CHD event (MI, stroke, or positive coronary angiogram), 31 individuals with diabetes mellitus, and 17 individuals with missing data at baseline or follow-up were removed from the analysis. The final sample consisted of 605 ECAC study participants, of whom 407 (67.3\%) had follow-up CT examinations.

**Risk Factor Assessment**

During the baseline and follow-up interview, participants reported current medication use and history of smoking, physician-diagnosed hypertension, MI, angiographic evidence of a blocked coronary artery, stroke, or diabetes mellitus. Height was measured by a wall stadiometer, weight by electronic balance, and body mass index calculated (kg/m\(^2\)). Waist circumference was measured at the umbilicus and abdominal obesity defined as waist circumference >102 cm in men and >88 cm in women.\(^{28}\)

Standard enzymatic methods were used to measure total cholesterol, high-density lipoprotein cholesterol, and triglycerides after overnight fasting.\(^{29,30}\) Systolic blood pressure and diastolic blood pressure levels were measured in the right arm with a random-zero sphygmomanometer (Hawksley and Sons). Three measurements at least 2 minutes apart were taken, and the average of the second and third measurements used for the analyses are presented here.

Individuals were considered hypertensive if they reported a prior diagnosis of hypertension and use of prescription antihypertensive medication, or if the average systolic blood pressure or diastolic blood pressure was ≥140 mm Hg or ≥90 mm Hg, respectively. Participants were considered diabetic if they reported using insulin or oral hypoglycemic agents, or if they reported a physician diagnosis of diabetes mellitus but were not currently taking a pharmacological agent to control their high glucose levels.

**Genotyping of MGP**

The Thr83Ala polymorphism in MGP was a priori chosen because this missense mutation could potentially elicit functional change in the protein; no other MGP SNPs were genotyped. Genotyping of the MGP Thr83Ala polymorphism was performed by subjecting 20 ng aliquots of genomic DNA to polymerase chain reaction amplification and subsequent restriction-endonuclease digestion. DNA was extracted from blood drawn at the time of the baseline physical examination. The oligonucleotide primers used were forward primer: ATTCATCTGAATTTGGCCTC and reverse primer ATTTCAAGAATGCTGCTACAG (Gibco BRL). The fragments were visualized by the use of ethidium-bromide staining after electrophoresis on 10% vertical acrylamide gels. Samples originally scored as heterozygotes were subjected to successive enzyme digestions and rescored to minimize the possibility of mistypings due to incomplete digestions. Genotypes were scored separately by 2 trained laboratory workers, and any discrepant typings were subjected to examination by a third person.

**Measures of CAC**

CAC was measured with an Imatron C-100 or C-150 electron beam CT (EBCT) scanner (Imatron Inc, South San Francisco, CA). Protocols at baseline and follow-up were identical,\(^{31,32}\) and methods to account for variability by scanner were used as described elsewhere.\(^{33,34}\) A dual scan approach was used beginning in 1993. A scan run consisted of 40 contiguous 3-mm-thick tomographic slices from the root of the aorta to the apex of the heart. Scan time was 100 ms/tomogram. Electrocardiographic gating was used, and all images were triggered at end-diastole during 2 to 4 breath-holds. A radiological technologist scored the tomograms with an automated scoring system without knowledge of other CT examination results for the same participant.\(^{35}\) CAC was defined as a hyperattenuating focus within 5 mm of the midline of a coronary artery, ≥4 contiguous pixels in size, and having CT numbers >130 Hounsfield units throughout. Areas ≥1 mm\(^2\) for all CAC foci were summed to provide a measure of CAC quantity.

**Replication Cohort**

The first phase of the Genetic Epidemiology Network of Arteriopathy (GENOA) study of the Family Blood Pressure Program was conducted in Rochester, MN, between June 1996 and October 2000.\(^{36}\) Sibships with ≥2 members having essential hypertension diagnosed ≥60 years, along with any other available siblings regardless of their hypertension status, were recruited. Follow-up studies, which occurred between December 2000 and February 2004, included CT examinations of the heart.\(^{37}\) Individuals with a history of coronary revascularization and pregnant or lactating women were excluded. Identical CAC measurement protocols (including EBCT scanners used), as well as clinical and laboratory protocols, were used in the ECAC study and the GENOA study. There were 246 GENOA study participants who had a first CT examination of the heart as part of the ECAC study between 1991 and 1998 and had a follow-up CT examination of the heart between 2000 and 2004. None of these 246 GENOA study participants were in the RFHS and thus represent an independent cohort. GENOA participants were genotyped using the Affymetrix SNP Array 6.0.\(^{38}\) Although this array does not contain the MGP Thr83Ala polymorphism (rs4236), proxy SNPs were searched using SNP annotation and proxy search using the HapMap CEU population.\(^{39}\) Nine genetically identical (\(R^2=1\)) proxy SNPs (distance from rs4236 ranging 2320–32576 base pairs) were identified that were directly genotyped on the Affymetrix 6.0 platform.

**Statistical Analysis**

A significance level of 0.05 was used for all analyses (as only a single SNP was tested a priori, no adjustment for multiple testing was made); all tests were 2-sided. The MGP Thr83Ala polymorphism was tested for consistency with Hardy–Weinberg equilibrium in the sample of all 665 ECAC participants with MGP genotype data. As some individuals were members of the same sibship (612 singletons, 23 sibships of size 2, and 1 sibship of size 3), 1 member of each sibship was randomly selected and used to obtain expectations under Hardy–Weinberg equilibrium. Individuals with MGP genotyping...
data represent a subsample of the entire ECAC study cohort. We compared those with and without genotyping data using χ² tests for discrete traits and Wilcoxon nonparametric tests for continuous traits. The association between MGP genotype and baseline demographic or CHD risk factors was estimated using logistic regression for discrete covariates and linear regression for continuous covariates. A varying number of CAC quantity measures were available for each participant (maximum of 2 at baseline and 2 at follow-up): 60 participants had 1; 147 had 2; 185 had 3; and 213 had 4 CAC measures. For descriptive purposes, when 2 CAC measures at a single time point were available, the average was used to represent CAC quantity. To reduce non-normality, CAC quantity was log transformed after adding 1.

Generalized linear mixed models were fit to examine the cross-sectional association between MGP genotype and CAC quantity variation; and test whether MGP genotype was associated with the rate of change in CAC quantity with time. These models allowed us to use each scan run available at baseline and follow-up, rather than averaging multiple scans at each time point. The models included a random participant intercept (ie, baseline CAC quantity) and fixed effects for the covariates. All models assumed an additive relation between MGP genotype and CAC quantity and progression.

All models included baseline age, time since baseline examination, male sex, a time by male sex interaction term, and a time by baseline age interaction term as covariates. MGP genotype was included to determine whether there was a cross-sectional association between MGP and CAC quantity and a time by MGP genotype interaction variable added to determine whether MGP genotype was associated with CAC progression. We additionally adjusted for baseline CHD risk factors (ie, systolic blood pressure, current smoker, total cholesterol, high-density lipoprotein cholesterol, antihypertensive medication use, and abdominal obesity). We considered each 2-way interaction with time and retained any significant interaction term. For MGP by time interactions, parameter estimates were exponentiated and interpreted as the relative increase in CAC quantity per year since baseline.

Additionally, we refit the final model, stratified by presence or absence of detectable CAC at baseline, to examine whether MGP was associated with incident CAC quantity or proliferation of existing CAC quantity. To account for potential correlation among members of the same sibship, we refit all final models randomly selecting 1 individual per family. Inferences were the same.

Identical models were fit within the GENOA-Rochester replication sample. The proxy SNP closest to MGP Thr83Ala was chosen for primary analysis (rs6488724, distance=2320 base pairs).

## Results

The ECAC study group included 309 men and 296 women with mean age of 51.6±10.3 years at baseline. The prevalence of detectable CAC at baseline was 44% (265/605). Mean time between examinations was 10.3 years (range 6.2–13.7 years). Mean annual change in CAC quantity was 3.4±8.5 mm²/yr.

MGP Thr83Ala polymorphism genotype frequencies were consistent with expectations under Hardy–Weinberg equilibrium (χ²=3.82; P=0.051). There were no differences between those with and without MGP genotyping with respect to age at examination, male sex, or presence or quantity of detectable CAC at baseline (P>0.05 for all; data not shown). MGP genotype was statistically significantly associated with current smoking (P=0.004) and antihypertensive medication use.

### Table 1. Baseline Characteristics, and Baseline, Follow-Up and Change in CAC, in 605 ECAC Study Participants by MGP Genotype

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Thr83Thr (n=190)</th>
<th>Thr83Ala (n=322)</th>
<th>Ala83Ala (n=93)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>51.8 (9.5)</td>
<td>51.5 (10.2)</td>
<td>51.6 (12.3)</td>
<td>0.885</td>
</tr>
<tr>
<td>Men</td>
<td>92 (48.4%)</td>
<td>167 (51.9%)</td>
<td>50 (53.8%)</td>
<td>0.357</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>118.9 (14.8)</td>
<td>118.1 (16.8)</td>
<td>119.1 (15.7)</td>
<td>0.941</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>77.0 (8.4)</td>
<td>76.4 (9.5)</td>
<td>77.8 (9.5)</td>
<td>0.716</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>192.8 (41.2)</td>
<td>195.7 (36.6)</td>
<td>189.8 (36.8)</td>
<td>0.762</td>
</tr>
<tr>
<td>High-density lipoprotein cholesterol, mg/dL</td>
<td>44.9 (12.7)</td>
<td>45.5 (13.0)</td>
<td>45.6 (15.5)</td>
<td>0.594</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>88.3 (10.7)</td>
<td>88.7 (10.2)</td>
<td>89.3 (17.6)</td>
<td>0.518</td>
</tr>
<tr>
<td>Current smoker</td>
<td>14 (7.4%)</td>
<td>36 (11.2%)</td>
<td>18 (19.4%)</td>
<td>0.004</td>
</tr>
<tr>
<td>Hypertension</td>
<td>40 (21.1%)</td>
<td>54 (16.8%)</td>
<td>20 (21.5%)</td>
<td>0.787</td>
</tr>
<tr>
<td>Antihypertensive medication</td>
<td>16 (8.4%)</td>
<td>32 (9.9%)</td>
<td>17 (18.3%)</td>
<td>0.025</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.2 (4.8)</td>
<td>27.0 (4.5)</td>
<td>26.7 (4.6)</td>
<td>0.319</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>86.8 (12.7)</td>
<td>86.2 (13.1)</td>
<td>86.1 (12.0)</td>
<td>0.631</td>
</tr>
<tr>
<td>Abdominal obesity</td>
<td>35 (18.4%)</td>
<td>58 (18.0%)</td>
<td>13 (14.0%)</td>
<td>0.421</td>
</tr>
<tr>
<td>Detectable CAC at baseline</td>
<td>81 (42.6%)</td>
<td>142 (44.1%)</td>
<td>42 (45.2%)</td>
<td>0.828†</td>
</tr>
<tr>
<td>Detectable CAC at follow-up*</td>
<td>71 (56.4%)</td>
<td>144 (64.0%)</td>
<td>39 (69.6%)</td>
<td>0.046†</td>
</tr>
<tr>
<td>Baseline CAC area, mm²</td>
<td>25.8 (76.3)</td>
<td>22.3 (70.8)</td>
<td>20.4 (49.2)</td>
<td>0.833†</td>
</tr>
<tr>
<td>Follow-up CAC area, mm²*</td>
<td>36.2 (98.1)</td>
<td>51.8 (137.4)</td>
<td>53.6 (101.5)</td>
<td>0.070†</td>
</tr>
<tr>
<td>Average change in CAC area, mm²/yr†</td>
<td>2.4 (6.4)</td>
<td>3.8 (9.8)</td>
<td>3.8 (6.9)</td>
<td>0.065†</td>
</tr>
<tr>
<td>Time between baseline and follow-up examinations, y*</td>
<td>10.5 (1.5)</td>
<td>10.2 (1.4)</td>
<td>10.4 (1.5)</td>
<td>0.328†</td>
</tr>
</tbody>
</table>

Data are mean (SD) or n (%); probability value is from comparison across genotype. Ala indicates alanine; BP, blood pressure; CAC, coronary artery calcification; MGP, matrix Gla protein; and Thr, threonine.

*407 participants had follow-up examinations (96 participants with Ala83Ala genotype, 225 participants with Thr83Ala genotype, and 126 participants with Thr83Thr genotype).

†Defined as follow-up–baseline CAC area/time between examinations (in years).

‡Adjusted for men and age at examination; average change in CAC area additionally adjusted for baseline CAC quantity; CAC quantity variables log transformed.
Model II†

Model II†

Model II†

(P=0.025) at baseline. There were no other statistically significant differences with respect to baseline demographic or risk factor variables (Table 1).

MGP Genotype and CAC Quantity and Progression

MGP genotype was not associated with cross-sectional CAC quantity (P=0.603) after adjusting for male sex, baseline age, time between examinations, a time by male sex interaction term, and a time by baseline age interaction term (data not shown).

MGP genotype was significantly associated with rate of change in CAC quantity (Table 2). For every 1 Ala83 allele increase, there was a faster rate of change in CAC quantity. In the fully adjusted model, every 1 Ala83 allele increase was associated with an estimated 1.9% (95% confidence interval, 0.7%–3.0%) per year since baseline larger increase in CAC quantity.

MGP Genotype and CAC Progression

Stratified by Detectable CAC at Baseline

Among those with detectable CAC at baseline, there was no evidence that MGP genotype was associated with CAC progression (P=0.159; Table 3). In the fully adjusted model, among those without detectable CAC at baseline, CAC progression was statistically significantly associated with MGP genotype (P=0.008; Table 3). Among those without detectable CAC at baseline, every additional Ala83 allele was associated with a 2.2% (95% confidence interval, 0.6%–3.9%) per year increase in CAC quantity.

Replication

Table I in the online-only Data Supplement provides demographic and clinical summary data on the GENOA-Rochester cohort used for replication. A total of 246 (116 men) white participants had a total of 944 CAC measures used in the replication analysis. Genotype frequencies for the proxy SNP for MGP Thr83Ala in the GENOA-Rochester population were similar to those reported previously in the GENOA cohort (P=0.754; data not shown). rs6488724 was not associated with CAC progression among unrelated GENOA participants. rs6488724 was not significantly and positively associated with CAC progression, but not with cross-sectional CAC (P=0.025; data not shown).

Discussion

In the current study, the MGP Thr83Ala polymorphism was associated with CAC progression, but not with cross-sectional CAC quantity. CAC progression is heritable, and there is evidence that unique genes are involved in variation in the quantity and progression of CAC.8 Thus, the Thr83Ala polymorphism in MGP seems to be involved in some, but not all, of the processes involved in the pathogenesis of CAC.

MGP gene expression can be induced by vascular smooth muscle cells in response to increased extracellular calcium concentrations46 and may be induced in response to atherosclerosis.37 The MGP Thr83Ala substitution may decrease the ability of MGP to bind calcium, leaving unbound calcium free to deposit in the arterial wall. Recently, MGP, in the uncarboxylated form, was found to be associated with...
Mechanistically, MGP interacts with other proteins in the calcification pathway.45 Thus future studies examining gene by gene interactions between MGP and other genes may be useful. For instance, there is evidence of an interaction between MGP T-138C polymorphism and osteopontin (OPN) T-443C polymorphism and presence of detectable CAC.13 Similarly, mice deficient in both OPN and MGP had twice greater arterial calcification than mice deficient only in MGP.46 OPN, like MGP, inhibits calcification.

The MGP Thr83Ala polymorphism is in strong linkage disequilibrium with other MGP polymorphisms.15 The association between MGP Thr83Ala and CAC progression may be attributable to a closely linked polymorphism; for example, a promoter polymorphism in MGP (MGP-7), in linkage disequilibrium with the Thr83Ala polymorphism, was associated with accelerated progression of atherosclerosis among patients with end-stage renal disease.47 We assessed the potential impact of Thr83Ala substitution on the MGP protein product using the sorting intolerant from tolerant (SIFT) algorithm,48,49 which uses sequence conservation across species to make predictions as to which amino acid substitutions are likely to be tolerated or damaged. The SIFT result shows that the Thr83Ala substitution is predicted to be tolerated. Similarly, the PolyPhen50 method did not predict the substitution to be probably damaging, instead assigning the possibly damaging category. Although these bioinformatic results suggest that this substitution may not substantively alter MGP protein function, there is no definitive experimental data assessing the function of this missense variant.

Using GENOA-Rochester as our replication cohort provides an independent cohort from the same underlying population as the ECAC cohort. Time between CT scans is associated with CAC progression;51 both cohorts had a similar length of time between scans, allowing for harmonization of the phenotype between the 2 studies. The proxy SNP chosen, rs6488724, seems to reside on a promoter polymorphism in MGP (MGP-7), in linkage disequilibrium with other MGP polymorphisms.15 The association between MGP Thr83Ala polymorphism and presence of detectable CAC.13 Similarly, mice deficient in both OPN and MGP had twice greater arterial calcification than mice deficient only in MGP.46 OPN, like MGP, inhibits calcification.

MGP Thr83Ala polymorphism and CAC progression may actually be stronger.

In summary, MGP is a candidate gene for increased CAC progression among white individuals free of overt CHD. The previously reported relation between the MGP Thr83Ala polymorphism and risk of MI may be attributable to faster CAC progression.

Table 4. Progression of CAC Quantity Was Modified by MGP Proxy SNP rs6488724 Genotype (Additive Model for Increase in A allele) in 246 GENOA-Rochester Study Participants (Replication Sample)

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Model I*</th>
<th></th>
<th>Model II†</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Time, y</td>
<td>β±SE</td>
<td>P Value</td>
<td>β±SE</td>
<td>P Value</td>
</tr>
<tr>
<td>rs6488724</td>
<td>−0.10±0.01</td>
<td>0.486</td>
<td>−0.10±0.15</td>
<td>0.492</td>
</tr>
<tr>
<td>Time×rs6488724</td>
<td>0.03±0.01</td>
<td>0.011</td>
<td>0.03±0.01</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Linear mixed effects model results for log(CAC Quantity+1). β indicates parameter estimate; CAC, coronary artery calcification; MGP, matrix Gla protein; and SNP, single nucleotide polymorphism.

*Adjusted for sex, baseline age, time between examinations, a time by age interaction term, and a time by sex interaction term.
†Additionally adjusted for systolic blood pressure (SBP), a time by SBP interaction term, smoking status, a time by smoking status interaction term, total cholesterol, high-density lipoprotein cholesterol, antihypertensive medication use, and abdominal obesity.

Microcalcifications in preatheroma atherosclerotic lesions, shifting to the carboxylated (active) form as lesion severity increased.16 Similarly, the association of MGP with CAC may vary by the state of phosphorylation or carboxylation of MGP, as recently demonstrated in a sample of 200 healthy women, with the state of MGP potentially varying by the underlying presence of CAC.19 Further, MGP expression is repressed in senescent vascular smooth muscle cells, providing a mechanism by which atherosclerotic plaque development may be due to appearance of vascular smooth muscle cells.40 MGP is also an inhibitor of hydroxyapatite crystal growth.42 Others have speculated that MGP may behave differently in the presence of atherosclerosis.45 Thus, there is biological plausibility for a role of MGP in not only the pathogenesis of CAC but also in the timing of its involvement in CAC development (i.e., incidence and growth of CAC).

Our finding that MGP ThrAla83 genotype (or its proxy SNP rs6488724) was statistically significantly associated with progression among those without detectable CAC at baseline, but not among those with detectable baseline CAC, is consistent with these preliminary pathophysiological findings. Additionally, in the Multi-Ethnic Study of Atherosclerosis, although most traditional risk factors were associated with both incident CAC and CAC progression, some traditional risk factors were associated with only 1 measure. For instance, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol were associated with incident CAC but not CAC progression.43 Atherosclerosis develops and progresses via various mechanisms.44 Thus further study of the underlying mechanism of atherosclerosis progression in the presence or absence of preexisting atherosclerotic disease is needed.

The previously reported increased risk of MI associated with MGP Thr83Ala15 may be a result of accelerated progression of coronary artery atherosclerosis. Individuals with a history of MI were excluded from the current analyses, and individuals requiring invasive cardiac revascularization procedures were ineligible for participation in the ECAC Study. There may be an underrepresentation of Ala83 in the current study, and the association between
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Disclosures
None.

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http://atvb.ahajournals.org/content/suppl/2013/01/10/ATVBAHA.112.300491.DC1

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Supplemental Material

Supplemental Table I. Baseline characteristics, and baseline, follow-up and change in CAC, in 246 GENOA-Rochester participants by rs6488724 genotype; data are mean (standard deviation) or n (%) and P-value is from comparison across genotype class.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>rs6488724 (n=110)</th>
<th>rs6488724 (n=92)</th>
<th>rs6488724 (n=44)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58.5 (8.8)</td>
<td>61.1 (7.1)</td>
<td>61.3 (7.8)</td>
<td>0.058</td>
</tr>
<tr>
<td>Male</td>
<td>57 (51.8%)</td>
<td>46 (50.0%)</td>
<td>13 (29.6%)</td>
<td>0.080</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>128.3 (16.7)</td>
<td>130.7 (17.9)</td>
<td>130.3 (14.8)</td>
<td>0.871</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>80.3 (8.2)</td>
<td>79.5 (9.8)</td>
<td>79.1 (9.3)</td>
<td>0.979</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>213.8 (33.0)</td>
<td>212.1 (31.8)</td>
<td>213.1 (35.0)</td>
<td>0.648</td>
</tr>
<tr>
<td>High-density lipoprotein cholesterol (mg/dL)</td>
<td>51.1 (14.3)</td>
<td>54.9 (16.7)</td>
<td>51.9 (17.2)</td>
<td>0.697</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>93.8 (11.8)</td>
<td>98.4 (23.7)</td>
<td>106.6 (29.9)</td>
<td>0.002</td>
</tr>
<tr>
<td>Current smoker</td>
<td>7 (6.4%)</td>
<td>4 (4.4%)</td>
<td>4 (9.1%)</td>
<td>0.636</td>
</tr>
<tr>
<td>Hypertension</td>
<td>73 (66.4%)</td>
<td>65 (70.7%)</td>
<td>36 (81.8%)</td>
<td>0.229</td>
</tr>
<tr>
<td>Anti-hypertensive Medication</td>
<td>63 (57.3%)</td>
<td>60 (65.2%)</td>
<td>34 (77.3%)</td>
<td>0.101</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>30.0 (5.8)</td>
<td>28.8 (5.0)</td>
<td>29.8 (5.8)</td>
<td>0.596</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>99.0 (14.6)</td>
<td>95.9 (15.4)</td>
<td>96.2 (16.2)</td>
<td>0.434</td>
</tr>
<tr>
<td>Abdominal Obesity</td>
<td>58 (52.7%)</td>
<td>48 (52.2%)</td>
<td>24 (54.6%)</td>
<td>0.875</td>
</tr>
<tr>
<td>Detectable CAC at baseline</td>
<td>75 (68.2%)</td>
<td>57 (62.0%)</td>
<td>28 (63.6%)</td>
<td>0.286†</td>
</tr>
<tr>
<td>Detectable CAC at follow-up</td>
<td>89 (80.9%)</td>
<td>68 (73.9%)</td>
<td>37 (84.1%)</td>
<td>0.876†</td>
</tr>
<tr>
<td></td>
<td>Baseline CAC area (mm$^2$)</td>
<td>Follow-up CAC area (mm$^2$)</td>
<td>Average change in CAC area (mm$^2$/year)*</td>
<td>Time between baseline and follow-up examinations (years)</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-----------------------------</td>
<td>-----------------------------</td>
<td>------------------------------------------</td>
<td>---------------------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>50.2 (115.5)</td>
<td>82.5 (148.6)</td>
<td>9.3 (13.0)</td>
<td>3.6 (1.3)</td>
</tr>
<tr>
<td></td>
<td>42.4 (84.4)</td>
<td>65.4 (111.7)</td>
<td>6.5 (11.7)</td>
<td>3.7 (1.2)</td>
</tr>
<tr>
<td></td>
<td>74.0 (162.6)</td>
<td>113.4 (216.1)</td>
<td>11.3 (18.8)</td>
<td>3.8 (1.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.201$^+$</td>
</tr>
</tbody>
</table>

CAC, coronary artery calcification; BP, blood pressure

*Defined as (follow-up - baseline CAC area)/time between examinations (in years)

$^+$Adjusted for male sex and age at examination; average change in CAC area additionally adjusted for baseline CAC quantity; CAC quantity variables log-transformed.