

Matrix Gla Protein Is Associated With Risk Factors for Atherosclerosis but not With Coronary Artery Calcification

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Objectives—Atherosclerotic coronary artery calcification (CAC) is associated with increased coronary heart disease (CHD) risk. Matrix Gla protein (MGP) is an inhibitor of calcification in vivo. However, little is known regarding the distribution of circulating MGP and its associations with CHD risk factors or with CAC in humans.

Methods and Results—Serum MGP concentrations were determined in 2 independent populations of men and women free of clinically apparent cardiovascular disease: study A, n=316, mean age 58 years, and study B, n=452, mean age 68 years. CAC was determined by computed tomography. Mean MGP concentrations were 98.4 and 198 ng/mL in men, and 97.4 and 201 ng/mL in women, in study A and study B, respectively. In both cohorts, MGP levels were higher with increasing age. In age-adjusted analyses, there was an association of circulating MGP with increasing Framingham CHD risk score (in study A, $P=0.003$ in men and $P=0.016$ in women, respectively; in study B, a nonsignificant increase in men and $P=0.05$ in women, respectively). Significant associations of circulating MGP with high-density lipoprotein and other individual CHD risk factors were also noted in both cohorts. There were no consistent associations between MGP and CAC after adjustment for CHD risk score in the 2 cohorts.

Conclusions—MGP is associated with individual CHD risk factors and the Framingham CHD risk score in men and women free of clinically apparent CHD. The relation of MGP with CAC deserves further study in larger populations. (*Arterioscler Thromb Vasc Biol.* 2006;26:2769-2774.)

Key Words: atherosclerosis ■ coronary artery calcification ■ coronary risk factors ■ matrix Gla protein

Ep^Epicardial coronary artery calcification (CAC) occurs in atherosclerosis¹ and is associated with increased risk for coronary heart disease (CHD).² It has been hypothesized that vascular calcification occurs via metabolic processes similar to those involved in bone deposition.^{3,4} However, human data are sparse to support or refute this hypothesis. As with other processes implicated in atherogenesis, such as lipid metabolism and inflammation, serum markers may provide clues to the underlying pathogenesis of CAC. In atherosclerosis, intimal macrophages and vascular smooth muscle cells express multiple proteins in association with calcification, including the vitamin K-dependent protein, Matrix Gla protein (MGP).⁵⁻⁷

Genetic and biochemical studies have established MGP as the first protein known to act as a calcification inhibitor in vivo. In mice, targeted deletion of the MGP gene causes rapid calcification of the elastic lamellae of the arterial media that begins at birth.⁸ In the rat, treatment with the vitamin K

antagonist, warfarin, at doses that inhibit the vitamin K-dependent γ -carboxylation of MGP, causes rapid calcification of elastic lamellae of arterial media and increased gene expression of MGP in the calcifying artery.⁹ Observational studies in small, selected cohorts of human subjects suggest that use of oral anticoagulants is associated with increasing valve calcification and CAC.^{10,11} These animal and human data support the hypothesis that MGP is an important regulator of vascular calcification.

The evidence for an association between serum MGP and vascular calcification is less consistent. There is a strong positive correlation between serum MGP concentrations and artery calcification in the rat, with 3-fold elevation in serum MGP in those animals with the greatest artery calcification,⁹ presumably secondary to increased local synthesis of MGP for the purpose of slowing the progression of artery calcification. However, increased serum levels of MGP, without a concomitant increase in MGP expression in the arterial walls,

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does not inhibit the ectopic mineralization observed in mice lacking MGP.¹² The available data in humans are conflicting, with serum MGP levels reported to be elevated in one study¹³ and decreased in another study¹⁴ of selected patients with severe atherosclerosis. It is possible that confounding factors may underlie these differing findings. However, the interrelationships between MGP and established atherosclerosis risk factors are unknown, and it is uncertain whether risk factors individually or together may confound the relationship between MGP and coronary atherosclerosis.

In the current study, we examined associations of serum concentrations of MGP with both coronary risk factors and CAC by computed tomography in 2 independent cohorts of men and women free of clinically apparent CHD: a community-based sample of middle-aged men and women (the Framingham Heart Study) and a sample of healthy elderly men and women participating in a vitamin K supplementation clinical trial.

Methods

Study Cohorts and Determination of Risk Factors

In the study A design, subjects were drawn from a stratified sample of participants from the Framingham Offspring Study enrolled in a pilot study of electron beam computed tomography (EBCT) and cardiac magnetic resonance imaging. The Offspring cohort was initially recruited in 1971 and consisted of 5124 men and women age 5 to 70 years.¹⁵ Of the 3219 participants attending the sixth examination cycle (1995 to 1998), we excluded from sampling 349 who had clinically apparent cardiovascular disease, 357 who lived outside New England, and 7 who were not between ages 35 and 84 years. The remaining 2506 subjects were stratified by sex, quartiles of age, and quintiles of Framingham CHD risk score. Those with Framingham CHD risk scores in the first and second quintiles were classified as low-risk, those in the third and fourth quintiles as medium-risk, and those in the highest quintile as high-risk. Subjects were sampled randomly and equally from each stratum, and invited to undergo EBCT, as previously described.¹⁶ Thirteen percent of eligible individuals contacted declined to participate; refusals were handled by randomly selecting another person from the same stratum.

The methods for anthropomorphic measurements, physician history, physical examination, and blood assays for cardiovascular risk factor information have been described.¹⁷ Data from the sixth examination cycle (1995 to 1998) were used for analyses of contemporary risk factors with CAC. The Framingham CHD risk score was calculated as previously described.¹⁸

Study B was comprised 452 men and postmenopausal women (mean age, 68 years; 267 women) participating in a randomized controlled trial of the impact of vitamin K supplementation on bone mineral density and CAC. Exclusion criteria included a usual vitamin K dietary intake $>90 \mu\text{g}/\text{d}$; a usual dietary calcium intake $>1500 \text{ mg}/\text{d}$; a usual dietary vitamin D intake $>1500 \text{ IU}/\text{d}$; women <5 years postmenopausal; femoral neck bone mineral density at screening >1.8 standard deviations above or below an age-matched reference mean; a 24-hour urine calcium to creatinine ratio $>300 \text{ mg}/\text{g}$ for women or $350 \text{ mg}/\text{g}$ for men; a terminal illness; renal or liver disease; a kidney stone in the past 5 years; current hyperparathyroidism; current oral anticoagulant use; current treatment with an osteoporosis treatment medication or estrogen replacement; known CHD; previous open heart surgery; and atrial fibrillation. All data presented here for study B were collected at the baseline visit, before randomization.

At the time of the baseline visit, information regarding medication use, medical history and smoking status were collected. Criteria used to define the presence of diabetes were the same as those for study A.¹⁷ Height and weight were measured while the subjects stood.

Body mass index was calculated as the weight in kilograms divided by the square of the height in meters. Blood pressure was measured in the right arm after the individual had been seated for at least 5 minutes. Current smokers were defined as subjects who reported smoking cigarettes on a regular basis during the previous year. Lipid concentrations were measured enzymatically, as described for study A.

Determination of Serum MGP

Blood samples from both studies were collected after fasting (>10 hours); serum samples were stored at -70°C for ≤ 2 years, and shipped on dry ice to University of California at San Diego for MGP analysis. Serum MGP was analyzed on first thaw using a radioimmunoassay.¹⁶ Because of the multiple post-translational modifications in the 79-aa residue MGP (5 residues of γ -carboxyglutamate and 3 of phosphoserine),^{19,20} the assay used MGP purified directly from human bone for assay standards and for preparation of the polyclonal antibody to MGP in rabbits.

Scanning and Analysis for CAC

Study A

EBCT scans were performed between 1998 and 1999 using an Imatron C-150 XP scanner in accordance with previously published protocols.¹⁶ Each scan was assessed by a technologist and over-read by a single experienced radiologist (M.E.C.), blinded to clinical data. A CAC score was generated using the method described by Agatston.²¹ Reproducibility was assessed by having 20 scans reread in a blinded fashion ($r=0.97$ for replicate readings). Image noise in each scan was assessed by determining the SD of pixel numbers in a region of interest within the aorta, as previously described.¹⁶

Study B

Scanning was performed using an 8-slice multidetector computed tomography scanner (Lightspeed Ultra; General Electric, Milwaukee, Wis) with prospective electrocardiographic gating during a single breath hold (12 seconds) using sequential data acquisition, as previously described.²² Each scan was assessed by a technologist and over-read by a single experienced radiologist (M.F. and U.H.). A CAC score was generated using a modification of the method described by Agatston.²¹

In study A, each participant provided additional written informed consent to undergo EBCT imaging, in addition to providing written informed consent before participation in each Framingham examination cycle. The imaging protocol was approved by the Boston University Human Studies committee and the Committee for Clinical Investigation of the Beth Israel Deaconess Medical Center. In study B, each participant provided written informed consent as approved by the Tufts-New England Medical Center and Massachusetts General Hospital Institutional Review Boards. The analysis of MGP in serum was approved by the University of California at San Diego Institutional Review Board.

Statistical Analyses

For both studies, we computed Pearson correlations for sex-specific relations of MGP levels with age, risk factors, and Framingham CHD risk score. We tested for the presence of significant associations between quartiles of MGP and the various risk factors using univariate analysis of variance and multiple linear regression. To adjust for the stratified sampling scheme, all analyses for CHD risk factors in study A were performed using analysis of variance (using the SURVEYREG and SURVEYMEANS procedures) in version 8.2 of SAS. For study B, all analyses for CHD risk factors were performed using analysis of variance (the general linear model procedure) in version 12.0 of SPSS. Tobit analysis was used in both studies to examine associations between MGP and CAC using SAS v8.2. Tobit analysis is recommended for the analysis of CAC because it minimizes the impact of extreme CAC scores on overall findings.²³ We conducted sex-specific analyses adjusted for age and then in addition for Framingham CHD risk score. A 2-sided $P<0.05$ was considered significant. A 2-sided $P<0.05$ was considered significant.

TABLE 1. Coronary Disease Risk Factors* by Quartiles of Serum Matrix Gla Protein (MGP), Study A

Men	Univariate ANOVA					Multiple Linear Regression	
	Q1 (lowest) (n=48)	Q2 (n=38)	Q3 (n=35)	Q4 (highest) (n=40)	P Trend	Unstd B	P Value
MGP, ng/mL*	72.2±1.3	88.5±0.5	103.4±1.0	134.8±2.9	<0.0001		
Age, years*	53.6±1.1	56.2±1.5	60.3±1.4	60.1±1.5	0.01	0.027	0.003
BMI, kg/m ²	28.5±0.6	28.6±0.6	30.0±0.6	30.1±0.7	0.036	0.021	0.095
Systolic BP, mm Hg	132.6±2.2	127.7±2.6	134.0±2.0	137.8±2.4	0.072	0.091	0.090
Triglycerides, mg/dL	173.9±16.1	134.7±12.6	153.5±14.1	163.9±18.4	0.78	0.170	0.743
Total cholesterol, mg/dL	219.4±4.2	210.0±5.14	198.6±6.73	197.0±4.9	0.0003	-0.159	0.166
HDL cholesterol, mg/dL	45.3±1.6	47.1±3.5	40.2±1.8	38.1±1.3	0.0008	-0.105	0.008
Total cholesterol/HDL, mg/mg	5.1±0.2	5.1±0.3	5.2±0.2	5.4±0.2	0.29	0.008	0.175
Diabetes, %	15.1	7.8	13.8	19.0	0.54	0.001	0.196
Current smoking, %	15.2	28.8	12.8	18.2	0.45	-0.0003	0.796
CHD risk score†	17.4±1.1	17.8±1.3	19.6±1.4	21.5±1.4	0.027	0.0270	0.003
Coronary calcification score ‡	263.8±53.0	335.0±108	296.6±87.2	316.7±73.7	—	1.109	0.524

Women	Univariate ANOVA					Multiple Linear Regression	
	Q1 (lowest) (n=40)	Q2 (n=35)	Q3 (n=41)	Q4 (highest) (n=39)	P trend	Unstd B	P Value
MGP, ng/mL*	64.3±1.2	82.3±0.7	98.4±0.6	145.0±9.1	<0.0001		
Age*	53.9±1.4	58.2±1.4	53.9±1.4	63.0±1.4	0.02	0.010	0.016
BMI, kg/m ²	26.6±0.7	29.6±1.5	28.4±1.13	29.3±1.3	0.18	0.018	0.352
Systolic BP, mm Hg	129.3±2.3	132.7±2.6	134.4±2.3	132.1±3.4	0.44	0.039	0.573
Triglycerides, mg/dL	145.5±11.6	142.1±12.8	171.1±29.7	163.8±15.7	0.22	0.138	0.044
Total cholesterol, mg/dL	209.2±5.5	218.6±7.5	218.7±4.8	228.1±5.5	0.0019	0.140	0.009
HDL C, mg/dL	60.3±2.4	54.9±2.3	54.0±2.1	49.8±2.4	0.005	-0.081	0.0001
Total cholesterol/HDL, mg/mg	3.7±0.2	4.2±0.2	4.3±0.2	4.9±0.2	0.0001	0.009	<0.0001
Diabetes, %	5.1	5.4	22.0	14.7	0.06	0.0002	0.577
Current smoking, %	10.4	21.4	6.5	18.0	0.76	0.0004	0.638
Postmenopausal, %	76.4	90.1	79.0	79.3	0.88	0.000	0.89
Current estrogen Use, %	46.9	37.3	15.3	8.1	<0.0001	-0.0025	0.006
CHD risk score†	7.5±0.9	10.2±1.2	11.0±1.1	10.9±0.9	0.007	0.010	0.016
Coronary calcification score‡	87.4±22.2	80.4±23.5	130.2±35.0	96.9±37.2	—	0.716	0.145

All values are presented as age-adjusted means±SEM unless otherwise indicated*.

†The Framingham Coronary Heart Disease Risk Score.¹⁸

‡Because of the skewness of coronary calcification, results are shown based on Tobit analysis.

BMI indicates body mass index; CHD, coronary heart disease; HDL, high-density lipoprotein; MGP, Matrix Gla protein.

Results

Study A

EBCT testing was performed on 327 participants, 316 of whom had MGP measurements (49% of whom were women). The mean±SD (range) ages were 57±9 (35 to 76) and 58±9 (35 to 77) years for men and women, respectively. Fasting MGP serum concentrations were significantly higher with increasing age in both men (P=0.003) and women (P=0.02), but did not differ between men and women in this population. The overall mean±SD (range) CHD risk scores were 17.3±12.1 (2 to 53) and 8.8±6.7 (1 to 27) for men and women, respectively. Baseline CHD risk factors in men and women are shown according to MGP quartiles in Table 1. In age-adjusted linear regression analyses for men, there was a statistically significant association of circulating levels of MGP with higher CHD risk score and lower high-density lipoprotein (HDL) cholesterol (Table 1). There was no

significant association of MGP with total or total:HDL cholesterol ratio. Among women, there were significant associations of circulating levels of MGP with higher CHD risk score as well as increasing triglycerides, total cholesterol and total:HDL cholesterol, and there was an inverse association with HDL cholesterol and use of estrogen replacement therapy (Table 1).

Using Tobit analysis, there was no significant association between MGP and CAC in age-adjusted analyses for women (P=0.15) or men (P=0.52) or after further adjustment for CHD risk score in either women or men.

Study B

Computed tomography (CT) testing was performed on 452 participants, 451 of whom had MGP measurements. The mean±SD (range) ages were 69±6 (60 to 81) and 68±5 (60 to 81) years for men and women, respectively. Fasting MGP

TABLE 2. Coronary Disease Risk Factors* by Quartiles of Serum Matrix Gla Protein (MGP), Study B

Men	Univariate ANOVA					Multiple Linear Regression	
	Q1 (lowest) (n=54)	Q2 (N=51)	Q3 (n=45)	Q4 (highest) (n=35)	P Trend	Unstd B	P Value
MGP, ng/mL*	153±2.3	185±2.3	216±2.5	266±2.8	<0.0001		
Age, years*	67.4±0.8	69.2±0.8	69.4±0.9	67.9±0.9	0.12	1.600	0.005
BMI, kg/m ²	27.6±0.6	26.8±0.6	38.3±0.6	29.9±0.7	0.01	2.237	0.002
Systolic BP, mm Hg	135.8±2.2	131.4±2.3	133.7±2.5	138.8±2.8	0.20	0.203	0.296
Triglycerides, mg/dL	106.8±9.9	113.4±10.1	114.2±10.8	135.2±12.2	0.34	0.104	0.018
Total cholesterol, mg/dL (n=137)§	188.5±5.0	191.1±5.0	202.5±5.6	202.1±6.4	0.16	0.211	0.063
HDL cholesterol, mg/dL	50.2±1.7	51.0±1.8	53.4±1.89	48.8±2.1	0.40	-0.191	0.456
Total cholesterol/HDL, mg/mg	3.9±0.1	3.7±0.2	3.8±0.2	4.2±0.2	0.17	6.381	0.044
Diabetes, %	5.6	5.9	2.2	2.9	0.76	6.833	0.666
Current smoking, %	13.0	3.0	2.2	2.9	0.08	-25.66	0.048
CHD risk score†	15.9±1.2	13.5±1.2	15.8±1.2	18.2±1.4	0.093	0.456	0.223
Coronary calcification score‡	255±90.9	488±90.0	330±97.1	343±109	—	0.324§	0.794‡

Women	Univariate ANOVA					Multiple Linear Regression	
	Q1 (lowest) (n=81)	Q2 (n=61)	Q3 (n=63)	Q4 (highest) (n=61)	P Trend	Unstd B	P Value
MGP, ng/mL*	147±2.3*	187±2.6	215±2.6	273±2.6	<0.0001		
Age, years*	66.2±0.5	68.2±0.7	68.6±0.6	69.5±0.7	0.002	2.372	<0.001
BMI, kg/m ²	26.1±0.6	28.2±0.7	27.8±0.7	30.0±0.7	0.001	2.259	<0.001
Systolic BP, mm Hg	127.7±1.9	128.4±2.2	132.6±2.2	132.3±2.2	0.22	0.383	0.034
Triglycerides, mg/dL	109.4±7.4	105.5±8.4	116.8±8.3	129.1±8.5	0.20	0.079	0.090
Total cholesterol, mg/dL (n=197)§	225.1±4.3	208.8±4.9	226.6±5.2	227.0±5.6	0.03	0.054	0.568
HDL cholesterol, mg/dL	64.8±1.7	60.0±1.9	60.8±1.9	55.9±2.0	0.01	-0.541	0.007
Total cholesterol/HDL, mg/mg	3.6±0.1	3.7±0.1	3.7±0.1	4.0±0.1	0.12	5.245	0.079
Diabetes, %	4.9	6.6	6.3	13.1	0.29	-17.44	0.133
Current smoking, %	8.6	6.6	1.6	1.6	0.13	-29.19	0.038
Coronary disease risk score†	9.0±0.7	8.7±0.7	9.6±0.08	11.1±0.8	0.11	1.001	0.054
Coronary calcification score‡	129.2±48.4	168.6±54.1	78.8±53.3	276.7±54.6	—	1.744§	0.030

All values are presented as age-adjusted means±SEM, unless indicated otherwise*.

†The Framingham Coronary Heart Disease Risk Score.¹⁸

‡Because of the skewness of coronary calcification, results are shown based on tobit analysis.

§Excluded those participants that reported cholesterol lowering medication use (n=69).

serum concentrations were higher with increasing age in both men ($P=0.005$) and women ($P<0.001$), but did not differ between men and women. The mean±SD (range) CHD risk scores were $15.6±8.7$ (2 to 40) and $9.6±6.0$ (2 to 32) for men and women, respectively. Baseline CHD risk factors are shown according to MGP quartiles in Table 2. In age-adjusted linear regression analyses for men, there was a statistically significant association of circulating MGP with higher body mass index, triglycerides, total:HDL cholesterol and current cigarette smoking (Table 2). Among women, there was a significant association of circulating MGP with increasing body mass index, systolic blood pressure, total:HDL cholesterol, cigarette smoking, and an inverse association with HDL cholesterol. For men, there was a nonsignificant association of MGP with higher CHD risk score and for women there was a borderline significant association ($P=0.05$).

Using Tobit analysis, there was a modest positive association between MGP and CAC in age-adjusted analyses for women ($P=0.03$) but not men (0.79), and this association remained significant after further adjustment for CHD risk score in women ($P=0.04$) but not in men ($P=0.79$).

Discussion

In these 2 study cohorts free of clinically apparent cardiovascular disease, we describe the distribution of circulating MGP concentrations and we report that MGP concentrations are associated with higher levels of a number of coronary risk factors, as well as the overall Framingham CHD risk score. In study A, subjects were drawn from a well-characterized, community-based cohort free of cardiovascular disease and sampled to represent a broad spectrum of ages and cardiovascular risk. In study B, subjects were older men and women selected for their low usual dietary vitamin K intake (<90 $\mu\text{g}/\text{d}$) to participate in a vitamin K supplementation study, and were also free of cardiovascular disease. To our knowledge, there are no other reports of associations between circulating concentrations of MGP and coronary risk factors in men and women free of clinically apparent CHD.

The findings of associations of MGP concentrations with HDL cholesterol and total:HDL cholesterol, and with Framingham CHD risk score in 2 independent studies provides consistent evidence that traditional lipid risk factors are

significantly associated with circulating MGP. In further analyses, there is no significant difference in high total cholesterol (> 240 mg/dL) or prevalence of total cholesterol-lowering drug treatment across MGP quartiles in either men or women (analyses not shown), suggesting that the predominant lipid association is with HDL cholesterol.

Increasing concentrations of MGP were modestly associated with higher levels of coronary calcium deposition after adjustment for CHD risk score in women but not men in the more elderly cohort study B. Our study sample sizes are relatively small, and estimates of association may be more reliable in larger sample sizes. Further research is justified in larger prospective cohorts to confirm associations with specific vascular risk factors and to assess the magnitude of association, independence from other risk factors, and sex-specificity of the positive relationship of MGP with vascular calcium deposits.

Increased concentrations of circulating MGP have been associated with arterial calcification in the rat⁹ and in patients with severe atherosclerosis.¹³ MGP is found at high levels in the vicinity of calcium deposits in mice and humans.²⁴ In humans, polymorphisms in the MGP gene have been associated with MGP promoter activity and circulating serum concentrations of MGP,²⁵ and evidence from one study suggests that variants in the MGP gene may be linked to CHD and CAC,²⁶ although in another study the associations with CAC are weak and not statistically significant.²⁷ Taken together, these findings suggest that arterial calcification may lead to increased MGP expression, perhaps in a feedback attempt to physiologically reduce bone-like formation of calcium deposits in the artery. Conversely, a more recent, small study attributed an inverse association between circulating MGP concentrations and coronary calcification to poor overall vitamin K status.¹⁴ These conclusions were not consistent with our observations in either cohort. Of note, both study A and study B were conducted in subjects free of CHD, likely at substantially lower risk than subjects in the previous study. It will be of interest to examine the role of randomization to vitamin K supplementation to progression of CAC and to change in MGP levels in study B, which is ongoing. Finally, increased serum levels of MGP, without concomitant increased MGP expression in the arterial walls does not inhibit the abnormal mineralization observed in mice lacking MGP.¹² Our finding of an inconsistent association of MGP with CAC in women, and no consistent association in men suggests that serum MGP concentrations are not robust in their predictive value of CAC, and that confounding by risk factors may explain much of the association.

Several limitations of these studies deserve consideration. MGP and risk factors were measured at the same time, but there was a time interval of ≈ 2 years between MGP measurement and EBCT testing in Study A. This might have led to an underestimation of the true magnitude of association between MGP and CAC score. However, the findings of study A are consistent with study B, in which MGP concentrations were determined at the same time as the CT scan. Image noise is correlated with body mass index in CT scans ($r=0.8$ in study A) and may confound coronary calcium readings in obese individuals. We found that body mass index

and image noise are highly correlated, and in study A, we conducted further analyses of associations between MGP and body mass index and found no significant association between MGP and body mass index ($P=0.14$). Subsequent analyses of the association of MGP and CAC, adjusting for body mass index, did not show any marked confounding of the association. Both studies were conducted in primarily white populations, so the findings may not be generalizable to non-white populations.

In our community-based cohorts, MGP levels are higher with increasing age and are associated with higher levels of individual coronary risk factors in middle aged and older men and women. Larger prospective cohorts need to be studied to confirm the strength of an independent positive relationship of MGP with vascular calcium deposits in women.

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Disclosures

None.

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