Introduction

Calcification of the coronary arteries is highly correlated with coronary atherosclerosis. The severity of coronary artery calcification (CAC), as assessed by electron-beam computed tomography (EBCT), is closely related to atherosclerotic plaque burden and cardiac event rate (1,2). CAC has been considered a useful noninvasive marker of coronary artery disease. However, the mechanism of vascular calcification is less well understood. Several studies have indicated that vascular calcification is an

Matrix Gla protein is associated with coronary artery calcification as assessed by electron-beam computed tomography

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Summary

Matrix Gla protein (MGP) is an extracellular matrix protein with wide tissue distribution. It has been demonstrated that the expression of MGP is detected not only in the normal blood vessels but also calcified atherosclerotic plaques, and that MGP deficient mice develop extensive arterial calcification. MGP is thought to be a regulator of vascular calcification. A recent clinical study demonstrates the association between polymorphisms of the MGP gene and increased risk of myocardial infarction. However, there are no reports of the relationship between serum MGP levels and coronary artery calcification (CAC). We evaluated the severity of CAC using electron-beam computed tomography (EBCT), and measured serum MGP levels by enzyme-linked immunosorbent assay in 115 subjects with suspected coronary artery disease. CAC scores were correlated with traditional risk factors, such as age, gender, hypertension, diabetes, hyperlipidemia and smoking. The serum MGP levels were lower in patients with CAC than in those without CAC (p<0.001). As the severity of CAC increased, there was a significant decrease in serum MGP levels. Serum MGP levels (U/L) were 116.7 ± 20.3, 104.9 ± 19.2, 95.2 ± 15.2 and 82.2 ± 19.7, (medians 115.5, 105.0, 94.8, and 81.9) for the subjects with normal (CAC score=0), mild (CAC score=1 to 99), moderate (CAC score=100 to 400), and severe (CAC score >400) coronary calcification, respectively. We found that serum MGP levels are inversely correlated with the severity of CAC. These data suggest a possible role for MGP in the development of vascular calcification.

Keywords

Matrix Gla protein, coronary artery calcification, atherosclerosis, vitamin K-dependent factors
actively regulated process similar to osteogenesis, and bone-associated proteins may be involved in the development of vascular calcification (3).

MGP, an extracellular matrix protein originally isolated from bone tissue, belongs to a family of proteins that contain γ-carboxyglutamate (Gla) residues (4). MGP immunoreactivity is detected not only in normal blood vessels but also in calcified atherosclerotic plaques (5). Moreover, it has been reported that MGP-deficient mice develop diffuse calcification of arteries and cartilage (6). It is likely that MGP plays an important role in the development of vascular calcification. Recent clinical studies showed that MGP polymorphisms were associated with an increased risk of myocardial infarction and vascular calcification of femoral arteries (7). However, an association between serum MGP levels and CAC has not been reported. In this study, we evaluated the severity of CAC with EBCT, and examined whether serum MGP levels are associated with coronary calcification.

Methods and materials

Patients
This study involved 115 patients with stable chest pain and/or signs of myocardial ischemia on exercise electrocardiography. Coronary risk factors, including total cholesterol, systolic blood pressure and body mass index (BMI), were measured at the time of a physical examination. Age and history of myocardial infarction, stroke and cigarette use were assessed through an interview preceding the physical examination. Hypertension was defined by systolic blood pressure of ≥140 mmHg, diastolic blood pressure of ≥90 mmHg, or the current use of antihypertensive medications. Diabetes was considered present if a patient was treated with insulin or oral agents, or had a fasting glucose level of ≥126 mg/dL (7.0 mmol/L). Hyperlipidemia was defined as total cholesterol level of ≥240 mg/dL (6.2 mmol/L), the current use of lipid-lowering treatment, or both. Patients with renal disease (serum creatinine levels >1.5) and bone metabolic disease such as osteoarthritis and osteoporosis, and patients receiving hormone replacement therapy or warfarin treatment were excluded from the study. Written informed consent was obtained from all patients.

Coronary artery calcification
All patients underwent EBCT imaging with an Imatron C-100 scanner. Images were obtained with 100-ms scan time and 3-mm single-slice thickness, with a total of 40 slices starting at the level of the carina and proceeding to the level of the diaphragm. Tomographic imaging was electrocardiographically triggered to 80% of the R-R interval. CAC was defined as a plaque of ≥4 consecutive pixels (area =1.37 mm²) with a density of ≥130 HU. Quantitative CAC scores were calculated according to the method described by Agatston et al (8). Four categories of CAC scores were considered: normal (CAC score=0), mild (CAC score=1 to 99), moderate (CAC score=100 to 400) and severe (CAC score >400).

Measurement
Venous blood samples were collected in the fasting state. Blood samples were centrifuged at 1600 g for 15 min at 4°C, and serum was separated and stored at -80°C until use. The serum level of MGP was determined using a competitive enzyme-linked immunosorbent assay (VitaK Inc., Maastricht, The Netherlands) with a monoclonal antibody against human MGP as previously described (9). All samples were measured in duplicate and averaged. The lower limit of detection was 8.5 U/L. The intra- and inter-assay variations were 5.1 and 9.6%, respectively. High sensitivity testing for C-reactive protein (CRP) was performed according to the methods described by the manufacturer (Behring NA latex CRP; Behring Institute). The biochemical parameters, including total cholesterol, high density lipoprotein (HDL)-cholesterol, triglycerides, creatinine and glomerular filtration rate (GFR), were measured using an automated chemistry analyzer. The LDL-cholesterol level was computed with the formula of Friedwald, et al (10).

Statistical analysis
All data are presented as mean ± SD unless otherwise noted. Comparisons between groups for study variables were done using the unpaired Student’s t-test for normally distributed parameters and the Mann-Whitney U-test for non-normally distributed data. The relationships between continuous variables were evaluated by linear regression. Differences between the four groups according to the extent of CAC scores were analyzed by Kruskal-Wallis test and Dunn’s test for multiple comparisons. A multiple regression analysis was applied to identify independent determinants of CAC scores. The dependent variable was CAC scores. The independent variables were as follows: MGP, CRP, HDL cholesterol and LDL cholesterol. A value of P<0.05 was considered significant.

Results
This study consisted of 115 subjects and included 69 men and 46 women. The clinical characteristics of this study group are shown in Table 1. The mean CAC score was 397 ± 827, ranging from 0 to 6,234. The median value of CAC scores was 68, with an interquartile range (25th to 75th percentile) of 3 to 345. Coronary risk factors including age (p<0.01), male sex (p<0.01), hypertension (p<0.01), diabetes (p<0.05), hyperlipidemia (p<0.01), and current smoking (p<0.05) were significantly associated with the presence of CAC in this study. There was a positive correlation between CAC scores and CRP (p=0.004). CRP levels (mg/dl) were 1.49 ± 2.47, 1.79 ± 3.18, 2.55 ± 5.61 and 5.06 ± 14.27 for the subjects with none, mild, moderate, and
severe CAC, respectively. In univariate analysis, CAC scores were negatively correlated with HDL-cholesterol (p=0.002), but not correlated with LDL-cholesterol (p=0.35).

We measured serum MGP levels in all 115 subjects who underwent EBCT. The mean serum level of MGP was 99.2 ± 22.1 (U/L), ranging from 40.9 to 155.1. There were no significant associations between serum MGP levels and coronary risk factors, including age, male sex, hypertension, diabetes, hyperlipidemia, and smoking status. Serum MGP levels were not correlated with serum creatinine levels and GFR in this study. Serum MGP levels were significantly lower in patients with CAC (95.7 ± 20.8 U/L) than in those without CAC (116.7 ± 20.3 U/L; p<0.001). As the severity of CAC increased, there was a significant decrease in serum MGP levels. Serum MGP levels (U/L) were 116.7 ± 20.3, 104.9 ± 19.2, 95.2 ± 15.2 and 82.2 ± 19.7, (medians 115.5, 105.0, 94.8, and 81.9 and ranges 99.8 to 131.4, 93.5 to 113.7, 87.5 to 107.3, 70.2 to 96.7) for the subjects with normal, mild, moderate, and severe CAC, respectively. This correlation was apparent in both men and women (Fig. 1).

To determine the independent risk factors for CAC scores, CRP, parameters of lipid metabolism such as HDL- and LDL-cholesterol and serum MGP levels were analyzed by multiple regression analysis. Serum MGP levels were found to be independently associated with CAC scores, whereas CRP, HDL- and LDL-cholesterol were not correlated with CAC scores (Table 2).

### Discussion

In this study, we found that serum MGP levels were significantly decreased at increasing severity of CAC as assessed by EBCT. MGP gene expression has been found in human vascul-
lar endothelial cells and smooth muscle cells (VSMCs) (11), and its expression was shown to be regulated by various growth factors and hormones (12, 13). Wallin, et al. reported that MGP precursors are present in the endoplasmic reticulum of VSMCs, and that the arterial wall has the vitamin K dependent γ-carboxylation system (14), suggesting that MGP protein may be processed as a secretory protein in the vessel wall. Although the main sources of circulating MGP are unknown, part of the MGP produced in vessel wall may contribute to circulating MGP. We previously reported that the expression of MGP decreased during vascular calcification in an in vitro calcification model using bovine VSMCs, and that its expression was restored to the level of uncalfied control by inhibiting VSMC calcification with bisphosphonate (15). Proudfoot, et al. demonstrated that increased levels of MGP mRNA were associated with calcification in cultured human VSMCs, which spontaneously form calcified nodules (16). Furthermore, it has been shown that extracellular ionic calcium regulates the expression of MGP through a G protein mediated cation-sensing mechanism (17). These findings suggest that the expression of MGP is modulated in the development of vascular calcification. Presently, the vascular effect of MGP remains unclear. However, it has been shown that MGP deficient mice develop severe arterial calcification (6), and that MGP modulates an activity of bone morphogenetic protein-2 (18), the principal osteogenic growth factor, suggesting that MGP may act as an inhibitory factor for vascular calcification.

Vascular calcification is often encountered in advanced atherosclerotic lesions and has long been considered to be a passive, degenerative, and end-stage process of atherosclerosis and inflammation. However, recent evidence indicates that bone matrix proteins such as osteopontin, matrix Gla protein (MGP) and osteocalcin are expressed in calcified atherosclerotic lesions (19), and that calcium regulating hormones such as vitamin D3 and parathyroid hormone-related protein regulate vascular calcification in in vitro vascular calcification models based on cultured aortic smooth muscle cells (20, 21). These findings suggest that vascular calcification is an actively regulated process similar to osteogenesis, and that bone-associated proteins may be involved in the development of vascular calcification. In this study, we examined the association between MGP and vascular calcification, and serum MGP levels were found to be decreased as the severity of CAC increased. It has been reported that CAC score as assessed by EBCT is a sensitive marker of coronary artery disease (22). Although we could not examine the severity of coronary artery disease by coronary angiography in this population, it is likely that serum MGP levels reflect the severity of coronary artery disease as well.

Hale, et al. reported that there is a proteolytic cleavage of the C-terminal region in MGP isolated from human bone (23). After MGP synthesis, part of MGP may be degraded and its fragments may be set free in the circulation. It is likely that our assay detects both full-length and degraded MGP. MGP originating from human and bovine bone is one of the most insoluble proteins known. One possibility is that MGP may be bound to a soluble carrier protein and circulate in the blood. Recently, it has been reported that MGP forms a complex with another calcification-inhibiting protein known as fetuin-A (α2-Heremans Schmid glycoprotein) and mineral (calcium and phosphorus); this high molecular weight complex is detectable in serum (24). Fetuin-A levels in serum are lower in dialysis patients, a group known to have substantial CAC, than in healthy controls (25). In histological studies, high levels of MGP immunoreactivity are detected at the borders of calcification in human coronary artery (26). The development of calcium crystals in the vasculature may further decrease circulating MGP levels because the Gla residues in MGP have a high affinity for calcium phosphate and hydroxyapatite crystals (4).

Our findings differ from a previous report on a Dutch cohort showing that atherosclerotic patients had increased levels of circulating MGP (9). The difference between both studies may be dependent on the ratio of carboxylation in MGP. Since vitamin K consumption in the Japanese population is much higher than in Europe, one hypothesis is that in the Japanese cohort, MGP is produced in its carboxylated form and adsorbs to the vascular calcification, reducing MGP concentration in the circulation, whereas in the Western population mostly under-carboxylated form of MGP are produced. Although there is no data about the association between serum MGP levels and vitamin K status in this population, the subjects treated with warfarin, a vitamin K antagonist that inhibits the γ-carboxylation of MGP, were excluded from this study. The assay that we used does not discriminate between carboxylated and under-carboxylated MGP. Therefore, future studies are required to examine whether the degree of carboxylation in MGP is associated with the development of vascular calcification. Although the assessment in a wide range of cohorts in different geographic areas is needed, our findings suggest that MGP may be involved in the development of coronary calcification and serum MGP level may reflect certain stages of coronary artery disease.

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References