original article

No association between MGP rs1800802 polymorphism and stenosis of the coronary artery

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BACKGROUND AND OBJECTIVES: Matrix Gla protein (MGP) was originally isolated from bone but it is known to be expressed in several tissues including kidney, lung, heart, cartilage and vascular smooth muscle cells (VSMC) of the blood vessel wall. Since it inhibits calcification in subendothelial space of vessels thus, we evaluated the association of rs1800802(T>C) polymorphism and stenosis of the coronary artery.

DESIGN AND SETTING: Cross-sectional case-control.

SUBJECTS AND METHODS: One hundred eighty two subjects recruited on the basis of study protocol from who underwent coronary angiography. The controls (n=70) had normal coronary arteries (up to 5% stenosis). The patients (n=112) subdivided into three subgroups; single-vessel disease (SVD), two-vessel disease (2VD) and three-vessel disease (3VD) based on the number of stenosed coronary vessels (at least 50% stenosis). rs1800802 (T>C) polymorphism was determined by PCR-RFLP technique.

RESULTS: Genotype distribution was not significant between control and patient groups. In addition, there were no significant differences between rs1800802 (T>C) frequency and gender (*P*=.092), and also patient subgroups (one-, two- and three vessel disease) (*P*=.840).

CONCLUSION: We concluded that rs1800802 (T>C) polymorphism within the MGP promoter is not related to stenosis of the coronary artery.

Cardiovascular calcification refers to pathological calcium phosphate deposition in the blood vessels, myocardium, and cardiac valves. Clinical consequences of cardiovascular calcification depend on its extent and the organ affected.[1,2] In the last decade, a growing body of evidence indicates that vascular calcification is the result not only of passive calcium phosphate deposition on atherosclerotic vessels, but also of an active ossification process involving vascular structures.[3]

Extracellular calcification is a common and clinically significant component of a number of important human diseases including atherosclerosis and aortic valve stenosis. The concentrations of calcium and phosphate ions in mammalian extracellular fluids are sufficiently high to induce precipitation of apatite, yet widespread tissue calcification does not usually occur in health.[4,5] A role for extracellular matrix proteins has previously been proposed in the pathogenesis of arterial calcification in the setting of atherosclerosis.[6,7]

Arterial wall cells are thought to be capable, in some circumstances, of assuming an osteoblast-like phenotype that may involve the expression of extracellular matrix proteins, such as matrix Gla protein(MGP).[8] MGP is expressed in vascular smooth muscle cells (VSMCs) and in chondrocytes but not in osteoblasts, whereas osteocalcin is expressed in osteoblasts and odontoblasts only.[9] Osteocalcin is the most abundant gla protein synthesized in the skeleton, yet its deletion in mice failed to show impaired extracellular matrix mineralization (ECMM). The most striking is the finding of Dhore et al[8] which showed constitutive immunoreactivity of matrix Gla protein, osteocalcin, and bone sialoprotein in nondiseased aortas and the absence of bone morpho-

genetic protein (BMP)-2, BMP-4, osteopontin, and osteonectin in nondiseased aortas and early atherosclerotic lesions. When atherosclerotic plaques demonstrated calcification or bone formation, BMP-2, BMP-4, osteopontin, and osteonectin were upregulated. Interestingly, this upregulation was associated with a sustained immunoreactivity of matrix Gla protein, osteocalcin, and bone sialoprotein.[8] Matrix Gla protein is constitutively expressed by vascular smooth muscle cells and the current understanding is that it is constantly needed locally to actively prevent calcification.[9] A lack of matrix Gla protein, possibly via lower levels of gene expression (via promoter polymorphisms) could lead to calcification.[9]

MGP was originally isolated from bone, but it is known to be expressed in several tissues including kidney, lung, heart, cartilage and VSMC of the blood vessel wall. It is an 84-amino acid (approximately 12 kDa) protein that contains five γ-carboxy glutamic acid (Gla) residues.[10] The Gla residues in MGP and all other vitamin K-dependent proteins are produced by γ-carboxylation of certain glutamic acid residues by γ-carboxylase, and require a reduced form of vitamin K as a cofactor.[11] In atherosclerotic arteries, Gla-containing proteins may play an important role in clearing calcium phosphate (hydroxyapatite) as a consequence of the strong affinity of Gla residues for this compound.[12]

The importance of MGP to prevent calcification in soft tissues in vivo is well illustrated in the mgp knockout mouse model, which exhibits intense arterial calcification leading to vessel wall rupture and premature death, and in the Keutel syndrome, a rare human recessive disorder characterized by diffuse cartilage calcifications as a consequence of nonsense mutations of the MGP gene.[13] The MGP gene located on the short arm of choromosome 12 (12p12.3). Up to 90 polymorphisms for the MGP gene have been submitted in dbSNP (www.ncbi.nlm.nih.gov/snp). Some polymorphisms have suggested could potentially alter MGP function.[14,15] Transfection studies showed that the rs1800802 polymorphism has an important impact on in vitro promoter activity when transiently transfected into VSMCs. This polymorphism of matrix Gla protein promoter alters its expression but is not directly associated with atherosclerotic vascular calcification.[16] Some studies have showed that rs1800802 (T>C) alters binding of an activating protein-1 complex and is associated with altered transcription and serum levels of MGP.[14]

The associations between some MGP polymorphisms and myocardial infarction,[15] coronary artery calcification[17] and atherosclerotic vascular calcification[16] have evaluated in some studies but, the results were controversial and no study was found about the stenosis. rs1800802 (T>C) polymorphism in some population studies have been described in **Table 1**.The aim of this study was to evaluate the association between rs1800802 (T>C) SNP within the MGP gene promoter and extent of stenosis in coronary arteries

SUBJECTS AND METHODS

One hundred eighty-two subjects recruited on the basis of study protocol from who underwent coronary angiography between February 2010 and March 2011. The subjects with MI at the last three months, diabetes (FBS>120 mg/dL) and the ones with kidney and liver diseases were excluded from the study. The patients (n=112) subdivided into three subgroups; single-vessel disease (SVD), two-vessel disease (2VD) and three-vessel disease (3VD) based on the number

Table 1. rs1800802 (T>C) polymorphism in population studies.

Study	Population	A Significant association of rs1800802 with vascular calcification
Farzaneh-Far et al 2001[13]	Healthy Subjects (frequency of genotypes was studied)	No
Taylor et al 2005[7]	Younger African-American and non-Hispanic white (Black compared to White subjects)	No
Kobayashi et al 2004[15]	Autopsy cases from aorta compared to patients with suspected coronary artery disease	No
Herrmann et al 2000[14]	Myocardial Infarction cases compared to healthy individuals	No
Brancaccio et al 2005[3]	Chronic kidney disease patients compared to healthy controls	Yes
Crosier et al 2009[16]	Healthy, older men and women compare to placebo-controls	Yes (Men) No (Women)

of stenosed coronary vessels (at least 50% stenosis). Moreover, the controls (n=70) had normal coronary arteries (up to 5% stenosis). The clinical medications and demographic information of subjects were obtained through medical records.

Blood was drawn from all subjects after an overnight fast. Lipid profile including total cholesterol, triglyceride, HDL–cholesterol and other biochemical factors were measured by routine clinical methods. LDL-cholesterol was calculated by Friedewald formula.[18] Whole blood from subjects was collected in EDTA-containing caps and store rapidly at −80°. The genomic DNA was extracted from WBC using salting out method.[19] The rs1800802 (T>C) polymorphism were determined by PCR technique.[20] PCR reaction was performed with final volumes 25 µl; Mgcl2 (1.5 mM), Fast start Taq polymerase (1.5 U), genomic DNA (0.2 µg), F-primer (1µM; m5'-ATAATTATATTATTGGCACTGAACTAGCATTGGAACTTTTCCCAACC-3-3') and R-primer (1µM;5'-TTATAATATTCTGATTAGTCTGGGATTGATAGATTGGTCTAGGATTGAG-3'). The temperature cycles (n=20) were followed after incubation in 950C for 5 min (95°C for 30s, 62°C for 30s, 72°C for 30s and 72°C for 3min as final extention. Then, the PCR products were subjected to RFLP. The rs1800802 (T>C) does not have a digestion site so a BsrI digestion site was designed in F-primer. The PCR product was 472bp. When T was within rs1800802 polymorphic site, BsrI was able to digest the fragment and produced a 426bp fragment.The primers was designed so that the digested product could be separated on agarose gel. PCR products and digested fragments with BsrI were run on 3% agarose gels and visualized by UV transillumination following ethidium bromide staining (**Figure 1**).

Statistical analysis was performed using statistical software package (SPSS 18.0, Chicago). The quantitative parameters were reported as mean and standard deviation. The differences between groups were evaluated by *t* and χ^2 tests. ANOVA test was also used to determine the differences between subgroups. A multinomial logistic regression analysis was performed to evaluate potential factors of stenosis in coronary arteries. A *P* value less than .05 was considered to be significant.

RESULTS

In this study, 182 subjects (100 men and 82 women) were studied. Some characteristics of the patients and controls are shown in **Table 2**. Compared with the control group, the patient group was significantly older (*P*<.001). Our analyses revealed a higher significant LDL-Cholesterol (*P*<.001), total cholesterol (*P*<.001), triglyceride (*P*=.03) and systolic blood pressure (*P*=.011) among the patients as compared to controls. There were no significant differences in the serum levels of HDL-cholesterol (*P*=.963), BMI (*P*=0.118), and diastolic blood pressure (*P*=.077) between the both groups.

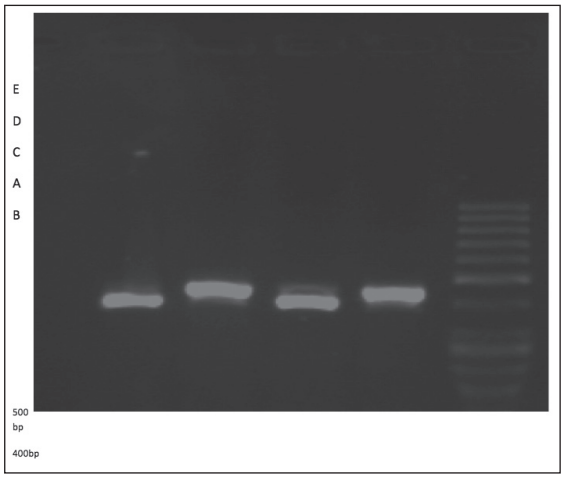

Figure 1. The PCR product digestion with BsrI. The PCR product was 472 bp. When T is in polymorphic site, BsrI digests the fragment and produced a 426 bp fragment(A and C).when C is in polymorphic site the 472bp fragment is intact(B and D). E is 50bp DNA marker.

Table 2. Characteristic of the study population.

Parameter	Control (n=70)	Patient (n=112)	*P* value
Sex (male/female)	23/47	77/35	.001
Age (year)	54.07 (16.97)	62.49 (11.74)	.001
Body mass index (kg/m^2)	26.61 (6.30)	25.21 (4.48)	.118
Systolic blood pressure (mmHg)	119.6 (34.08)	130.48 (22.89)	.011
Diastolic blood pressure (mmHg)	72.97 (22.27)	78.18 (17.05)	.077
Smoking (yes/no)	12/56	28/86	.34
LDL-cholesterol (mg/dL)	93.77 (34.20)	121.80 (26.81)	.001
HDL-cholesterol (mg/dl)	39.99 (12.36)	39.90 (11.68)	.963
Triglyceride (mg/dL)	159.74 (62.07)	195.47 (79.21)	.002
Total cholesterol (mg/dL)	156.83 (48.58)	184.93 (41.16)	.001

rs1800802 (T>C) genotype

The genotypes and allele frequencies showed nonsignificant differences in the control and patient groups;

Table 3. Genotype and allele frequencies in patient and control groups.

Allele/Genotype		Control (n=70)	Patient (n=112)	*P* value
Rs1800802				
Allele	T	84 (60.%)	111(49.5%)	.066
	C	56 (40%)	113 (50.5 %)	
Genotype	TT	34 (46.6%)	39 (53.4%)	
	TC	16 (32.7%)	33 (67.3%)	.183
	CC	20 (33.3%)	40 (66.7%)	

Table 4. Genotype distribution in patient subgroups.

Parameter	Patients (n=112)				*P* value
	SVD	2VD	3VD	All	
TT	8	11	20	39	
TC	9	6	18	33	.840
CC	8	11	21	40	

P=.183 and *P*=.066, respectively (**Table 3**). CC+TC versus TT were not significant in the patient group as compared with the control group and had no significant differences in subgroup of patients (data not shown). There were not a significant differences in genotype frequency in men and women (*P*=.092), and in patient subgroups (one-, two- and three vessel disease) (*P*=.840). The proportion of CC homozygote was higher in the 3VD subgroup as compared with the others, but the difference was not significant (**Table 4**). Moreover, the multinomial logistic analysis confirmed that the role of rs1800802 is not important in the extent of disease (**Table 5**). Sex has a significant association with SVD, 2VD (*P*=.036 and *P*=.001,respectively).A positive relation of LDL-C with 2VD and 3VD was observed (*P*=.040 and *P*=.000, respectively).

DISCUSSION

The expression of bone-related genes in atherosclerotic lesions was described over a decade ago.[21,22] Matrix Gla protein 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.[9,23-25] The promoter region of MGP contains nucleotide variations, especially rs1800802 (T>C) that can alter the expression level of MGP,[14] that are related to the occurrence of acute myocardial infarction in subgroup of patients as demonstrated by population-based extensive clinical studies.[15]

Moreover, the matrix Gla protein is an important inhibitor of vessel and cartilage calcification and could modulate plaque calcification and coronary heart disease risk. Since calcification is becoming an increasingly important medical problem caused coronary stenosis[26] and some studies have showed that rs1800802(T>C) polymorphism of MGP promoter alters its expression so, using a genetic approach, we evaluated the frequency of this polymorphism in patient and control groups and testing their possible association with the extent of stenosis.

The Coronary Artery Risk Development in Young Adults (CARDIA) study has shown that allele (C) was common in both white (0.39) and black (0.53) participants and the individual e?ect of the rs1800802 (T>C) polymorphism with coronary calcification was weak and not statistically significant.[7] The CC genotype was significantly common among Iranian subjects, (TT 40.1%, TC 26.92% and CC 32.96%) compared with that reported in Japan, Netherlands, Northern Ireland and France.[14-16]

It has been suggested that MGP is an important and potent inhibitor of vascular calcification in man.[27] Crosier et al found a gender specific association between rs1800802T>C and coronary artery calcification in older, healthy men and women of European descent.[3] Our data showed that the frequency of this polymorphism is not significantly different in men and women. Brancaccio et al[3] evaluated the genotype distribution in hemodialysis (HD) patients (in 26 patients with CKD stage 3) and in healthy controls. TT homozygote was more frequent in the HD group versus controls.

Moreover, in vitro analysis of MGP promoter activity revealed that the C allele reduced promoter activity by 20% in rat vascular smooth muscle cells and by up to 50% in a human fibroblast cell line. Despite the identification of a functional effect on MGP promoter activity in vitro, the C allele was not related to calcification, femoral artery atherosclerosis, or MI in their studies.[15] This result may indicate that the reduction in absolute levels of MGP production caused by the C allele may not be sufficient to affect these phenotypes. In agreement with other studies,[7,15,16] we did not find any significant difference in T>C frequency in patient group compared with control group. Kobayashi et al[16] reported that the C genotype (TC+CC) tended to show a higher calcification factor than the TT geno-

Table 5. Multinomial logistic regression analysis.

Parameters	Single vessel disease (SVD)		Two vessel disease (2VD)		Three vessel disease (3VD)	
	P value	OR (CI)	*P* value	OR (CI)	*P* value	OR (CI)
Age (year)	.371	1.020 (0.975-1.068)	.011	1.061 (1.013-1.110)	.160	1.028 (0.988-1.070)
Sex (female/male)	.036	0.298 (0.096-0.926)	.001	0.163 (0.055-0.486)	3.14E-07	0.069 (0.024-0.192)
LDL-C (mg/dL)	.075	1.026 (0.977-1.055)	.040	1.025 (1.001-1.051)	.000	1.042 (1.018-1.067)
Cholesterol (mg/dL)	.394	1.009 (0.987-1.031)	.721	0.996 (0.980-1.013)	.905	0.999 (0.983-1.014)
BMI (kg/m^2)	.760	1.106 (0.577-2.120)	.379	0.767 (0.424-1.386)	.516	0.836 (0.486-1.416)
SBP (mm Hg)	.635	1.007 (0.975-1.040)	.294	1.016 (0.985-1.047)	.032	1.030 (1.002-1.058)
DBP (mm Hg)	.904	1.002 (0.957-1.050)	.838	1.004 (0.959-1.051)	.218	0.975 (0.937-1.014)
Rs1800802	.760	1.106 (0.577-2.120)	.334	1.340 (0.739-2.432)	.076	1.629 (0.949-2.799)

type. We observed that distribution of CC+TC versus TT has no significant difference between the patients and controls and has no effect on the extent of stenosis.

Since no study was found on the association of rs1800802T>C polymorphism with the extent of stenosis, we evaluated this polymorphism in SVD, 2VD and 3VD subgroups of patient. The proportion of CC homozygote was higher in the 3VD subgroup as compared with the others, but the difference was not significant.

In conclusion, it should be borne in mind that numerous factors contribute towards the marked coronary artery stenosis, i.e. all the 'classic' risk factors for atherosclerosis. Hypertension, hyperglycemia, hyperlipidemia and especially aging are known to independently and strongly affect the stenosis of coronary artery so that, in our study some of these factors were significant. Thus, the contribution of promoter polymorphism rs1800802 may not be a potential factor to affect the stenosis of coronary artery.

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