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ORIGINAL ARTICLE

Analysis of *MMP-7* and *TIMP-2* gene polymorphisms in coronary artery disease and myocardial infarction: A Turkish case-control study

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Abstract Matrix metalloproteinase (MMP) and tissue inhibitors of metalloproteinase (TIMP) have a significant role in tissue remodeling related to cardiac function. In earlier studies, *MMP-7* A-181G (rs11568818), C-153T (rs11568819), C-115T (rs17886546), and *TIMP-2* G-418C (rs8179090) polymorphisms have been studied in various diseases. However, association between coronary artery disease (CAD) and these polymorphisms has been poorly studied. The goal of this study is to investigate the association of CAD and myocardial infarction (MI) with *MMP-7* or *TIMP-2* polymorphisms. This study included 122 CAD patients and 132 control individuals. DNA was extracted from whole blood. Polymerase chain reaction-restriction fragment length polymorphism and automated direct sequencing method were used for genotyping of these polymorphisms. No significant differences were found between *MMP-7* A-181G, C-115T, and *TIMP-2* G-418C polymorphism and CAD or MI in a Turkish population. Despite the fact that the genotypes of *MMP-7* C-153T polymorphism had no significant differences among MI and control groups, allele frequencies of C-153T polymorphism were significantly different between the two groups. Our study is the first report to clarify the appreciable relationship

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between *MMP-7* C-153T polymorphism and MI development in CAD patients. However, these findings also need to be confirmed in other populations so we can improve our knowledge about the genetic factors affecting the development of CAD.

Introduction

Atherosclerosis is the major cause of coronary artery disease (CAD) with stable and unstable periods, and myocardial infarction (MI) can develop in patients throughout the unstable periods in the vascular wall [1]. CAD accounts for more than 80% of sudden cardiac deaths, and the remaining 20% of deaths are caused by other diseases such as cardiomyopathies, left ventricular hypertrophy, and long QT syndrome [2].

According to earlier studies, alteration of the myocardial extracellular matrix (ECM) contributes to the progressive remodeling process [3]. ECM components may cause fragility of the plaque in addition to generation of atherosclerotic and restenotic lesions. Among ECM components, matrix metalloproteinases (MMPs) have a substantial role in initiating acute coronary syndrome [4]. MMPs are a significant family of metal-dependent enzymes and are responsible for ECM degradation [5]. MMP-7 (PUMP-1, matrilysin, E.C.3.4.24.23), an important member of the MMP family, is a protease with wide substrate specificity comprising fibronectin, elastin, type IV collagen, and proteoglycans [6—8]. Whereas active-MMP-7 is 19 kDa, Pro-MMP-7 is 28 kDa [9]. COOH-terminal hemopexin-like domain is not found in MMP-7 [10]. During the acute phase after MI, in particular, it might affect left ventricular remodeling [9]. In addition, it has been proposed that elevation of MMP-7 activity can play a substantial role in CAD [10].

The family of tissue inhibitors of metalloproteinase (TIMP) inhibits the proteolytic activity of MMPs, and it is composed of four members: TIMP-1, TIMP-2, TIMP-3, and TIMP-4 [11]. TIMP-1 potentially blocks the activity of most MMPs including MMP-7, MMP-9, but not MMP-2. However, TIMP-2 is a potent inhibitor of numerous MMPs, excluding MMP-9 as well. TIMP-3 binds MMP-1, MMP-2, MMP-3, MMP-9, and MMP-13, whereas TIMP-4 binds MMP-1, MMP-3, MMP-7, and MMP-9 [4]. Because balance between MMP and TIMP is important, alteration of the ECM compositions can contribute to alteration of myocardial structure or geometry [12]. Altered balance in the MMP—TIMP interaction has been shown to play a significant role in the development of many diseases, including tissue remodeling after cardiac infarction, arthritis, periodontitis, and pulmonary emphysema [13,14]. TIMP-2 expression increases cardiac fibroblast collagen synthesis. Additionally, TIMPs and MMPs pursue a spatiotemporal pattern during myocardium repairing after MI [15]. Earlier, a single nucleotide polymorphism (SNP) in promoter region of *TIMP-2* gene at position 418 has been identified by Hirano et al [16]. This alteration (G-418C, rs8179090), positioned in the consensus sequence of Sp1 binding region, can influence transcriptional activity [12,14,17]. We hypothesized that *TIMP-2* polymorphism might influence CAD, because balance between MMP and TIMP level is important in CAD.

According to a growing body of evidence, MMP gene polymorphisms in their promoters may have brought about variable MMP expressions in distinct individuals [6]. Of the polymorphisms in *MMP* genes, *MMP7* gene polymorphisms are associated with CAD, acute MI, multiple sclerosis, rheumatoid arthritis, and cancers as other MMP genes such as *MMP-9* and *MMP-2* [6,18]. The *MMP-7* gene is localized on chromosome 11q21—q22 and consists of two functional SNP in the promoter region. A-181G (rs11568818) and C-153T (rs11568819) polymorphisms, which are known to modulate the gene expression by affecting the interaction of nuclear binding proteins, have been shown to exert allele-specific effects on the activity of the their own promoters [7,18,19]. Moreover, the combination of −181G allele with −153T allele leads to higher gene expression [20].

The purpose of our study was to compare the distribution of *MMP-7* A-181G (rs11568818), C-153T(rs11568819), C-115T (rs17886546), and *TIMP-2* G-418C (rs8179090) polymorphisms in patients with CAD or MI and control individuals in the Turkish population.

Materials and methods

Study participants

Angiographically characterized 122 CAD patients and 132 controls were included in this study. All CAD patients had ≥50% stenosis in at least one coronary vessel. All samples were obtained from patients and controls admitted to Gazi University (Ankara, Turkey). Existence or absence of an MI history was described by integrating the clinical history data, after a medical records analysis including the typical MI sequelae, enzyme changes, and electrocardiogram on ventricular angiography. While collecting control samples, individuals with a history and clinical or instrumental proof of atherosclerosis in peripheral arteries were excluded from the study group. The controls had undergone coronary angiography following noninvasive cardiac examination because of atypical chest pain. Negligible coronary artery stenosis was left out in this study. All volunteers were from the Turkish population. All patients and controls were interviewed, and records on hypercholestrolemia, hypertension, diabetes mellitus, smoking habits, and family history of CAD were collected. According to the guidelines of the Gazi University of Ethics Committee, informed consent was obtained from all patients and controls. If the blood pressure of patients and controls exceeded 140/90 mmHg or they were given antihypertensive medication, they were described as hypertensive. Meanwhile, diabetes mellitus was diagnosed if the participants had a history of antidiabetic drug treatment, a previous diagnosis, or fasting glucose levels higher than 126 mg/dL. The patients were

evaluated to be hypercholesterolemic if their low-density lipoprotein levels were higher than 130 mg/dL.

Analysis of MMP-7 and TIMP-2 polymorphisms

Heliosis DNA extraction kit (Metis Biotechnology, Ankara, Turkey) was used for DNA extraction from whole blood samples according to the manufacturer's instructions.

DNA amplification was performed in a 50-μL reaction mixture including 200μM of each dNTP, 200mM $(NH_4)_2SO_4$, 75mM Tris—HCl (pH 8.8), Tween-20 (0.1%), 1 unit Taq DNA polymerase (Fermentas, Vilnius, Lithuania), various concentrations of $MgCl_2$, DMSO, and 50 pmol of primers for each polymorphism (Table 1). A previously published forward and reverse primer pair was used for the amplification of DNA region containing *TIMP-2* polymorphic site [21]. Forward and reverse primer pairs for three polymorphisms of *MMP-7* were newly designed by our group (Table 1). The polymerase chain reaction (PCR) cycle was fulfilled in a thermal cycler (Eppendorf, Hamburg, Germany). The PCR procedure was as follows: 94°C at 5 minutes following 30 cycles of 45 seconds for 94°C, 30 seconds for 55—65°C (Table 1), 1 minute for 72°C, and 5 minutes final extension for 72°C. In order to perform genotyping of *TIMP-2* G-418C polymorphism, PCR products (254 bp) were digested overnight at 37°C with 10 units of *Ava*I restriction enzyme (MBI, Fermentas, Vilnius, Lithuania) and then analyzed by 4% agarose gel electrophoresis. Gels were visualized and recorded via a Kodak Gel Logic 100 Imaging System (Kodak Co., Rochester, NY, USA).

To confirm the restriction fragment length polymorphism results for *TIMP-2* polymorphism on randomly selected samples and determine the *MMP-7* polymorphisms on all samples, nucleotide sequencing was performed via forward primers using ABI Prism 310 Genetic Analyzer (Applied Biosystems Divisions, Foster City, CA, USA). Spin PCRapid Kit (Invitek, Berlin, Germany) was used for purification of PCR products to analyze MMP-7 polymorphisms. Automated DNA sequencing was performed using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's guidelines.

Statistical analysis

SPSS Statistical Package (version 15.0) for Windows (SPSS Inc., Chicago, IL, United States) was used for statistical analysis. Genotype distribution of all polymorphism was calculated for deviation from the Hardy—Weinberg equilibrium (HWE) by using Chi-square (χ^2) tests (1 degree of freedom). Fisher's exact test (when expected values were less than 5) or χ^2 test was used to compare the allele and genotype frequencies in patients and controls. In order to estimate the relative CAD risk, crude odds ratios (ORs) was calculated with their 95% confidence intervals (CIs). Associations were confirmed by binary logistic regression analysis with backward LR logistic regression method. SNPStats was used to estimate *MMP-2* haplotype frequencies [22]. A p value of <0.05 was considered statistically significant. The statistical power was calculated using the software QUANTO 1.2 (website: http://biostats.usc.edu/software) [23].

Results

The baseline parameters of CAD patients and controls are demonstrated in Table 2. Our results have shown statistically significant variation between CAD with or without MI (CAD + MI) and control groups with regard to all demographic and clinical characteristics, but not diabetes mellitus and smoking habits. When compared to the control group, statistically appreciable variation in smoking habits, family history, hypertension, and hypercholesterolemia were observed in the CAD group. Moreover, statistically significant discrepancies were also determined in CAD with MI patients in terms of sex and smoking habit when compared to controls (Table 2).

The study had a power of >80 for *MMP-7* A-181G polymorphisms (95% power, OR = 2.0; mode of inheritance: log-additive). Other power values were <50 for *MMP-7* C-153T and *TIMP-2* G-418C polymorphisms as a result of having a very rare allele. In the cases and controls, the genotype frequencies of *MMP-7* A-181G, C-153T and *TIMP-2* G-418C polymorphisms did not diverge from the HWE (for cases, $\chi^2 = 0.02$, 0.05, and 0.03, respectively; for controls, $\chi^2 = 1.71$, 0.87, and 0.05, respectively; $p > 0.05$). The HWE of *MMP-7* C-115T polymorphism was not calculated in cases and control individuals carrying only the CC genotype.

In CAD + MI and control volunteers, the genotype and allele distribution of the *MMP-7* A-181G, C-153T, and *TIMP-2* G-418C polymorphisms are indicated in Table 3. Our results showed that AA and CC genotypes were frequently observed in cases and controls when comparing the genotype dispersion of the A-181G, C-153T polymorphisms,

Table 1 Oligonucleotide primers, restriction endonucleases, conditions used in PCR amplifications, RFLP fragments, and sequencing.

Polymorphism	SNP ID	Primer sequence	AT/MC/DMSO	PCR product	RE	RFLP fragments
MMP-7 −181 A/G	rs11568818	F: 5′-tgtcctgaatgatacctatgagag-3′	65°C/1mM	247 bp	—	—
MMP-7 −153 C/T	rs11568819	R: 5′-tactcagtggataaaggtgtaagct-3′				
MMP-7 −115 C/T	rs17886546					
TIMP-2 −418 G/C	rs 8179090	F: 5′-cgtctcttgttggctggtca-3′	62°C/2.5mM/3%	304 bp	*Ava*I	G = 230, 51, and 23 bp
		R: 5′-ccttcagctcgactctggag-3′				C = 253 and 51 bp

Amplifications of −181 A/G, −153 C/T, and −115 C/T polymorphisms were performed with a common forward and reverse primers under the same PCR conditions.
AT = annealing temperature; DMSO = dimethyl sulfoxide; MC = $MgCl_2$ concentration; PCR = polymerase chain reaction; RE = restriction endonuclease; RFLP = restriction fragment length polymorphism; SNP = single nucleotide polymorphism.

Table 2 Baseline characteristics of the patient with CAD/MI and control individuals.

Variable	CAD + MI			CAD			MI		
	Patients (122)	Control (132)	*p*	Patients (84)	Control (132)	*p*	Patients (38)	Control (132)	*p*
Age (y)	60.53 ± 11.3	57.56 ± 10.7	**0.034**	60.40 ± 10.8	57.56 ± 10.7	0.062	60.81 ± 12.4	57.56 ± 10.7	0.116
Sex (Male/female)	78/44	60/72	**0.003**	46/38	60/72	0.182	32/6	60/72	**0.0001**
Smoking habits (Smokers/ nonsmokers)	56/66	54/78	0.422	30/54	54/78	0.445	26/12	54/78	**0.003**
Hypercholesterolemia (with/without)	74/48	55/77	**0.002**	55/29	55/77	**0.001**	19/19	55/77	0.361
Hypertension (with/without)	77/45	57/75	**0.001**	54/30	57/75	**0.002**	23/15	57/75	0.059
Diabetes mellitus (with/without)	34/88	30/102	0.346	24/60	30/102	0.334	10/28	30/102	0.646
Family history (with/without)	45/77	29/103	**0.009**	34/50	29/103	**0.004**	11/27	29/103	0.372

p values <0.05 and <0.001 are shown in bold.
CAD = coronary artery disease; MI = myocardial infarction.

respectively. Based on the results, a significant relationship between these polymorphisms and CAD was not found ($p > 0.05$). As for the C-115T polymorphism, only the CC genotype was found in patients and controls; therefore, there were no statistically significant relation among cases and control ($p > 0.05$). In addition to *MMP-7* polymorphism, the GG genotype frequency of *TIMP-2* G-418C was higher in both groups, and there were no appreciable discrepancy in

Table 3 Genotype and allele frequencies of MMP-7 and TIMP-2 polymorphisms for CAD + MI patients and controls.

Genotypes	Controls ($n = 132$)	Cases ($n = 122$)	*p* Value	OR (95% CI)	*p*
MMP-7 −181 A/G			0.511		
AA	48 (36.4%)	37 (30.3%)		1[a]	
AG	57 (43.2%)	61 (50%)		1.39 (0.79−2.43)	0.251
GG	27 (20,4%)	24 (19.7%)		1.15 (0.57−2.32)	0.689
AG + GG[b]	84 (63.6%)	85 (69,7%)		1.31 (0.78−2.22)	0.308
Alleles			0.551		
A	153 (58%)	135 (55.3%)		1[a]	
G	111 (42%)	109 (44.7%)		1.11 (0.78−1.58)	0.551
MMP-7 −153 C/T			0.255		
CC	118 (89.4%)	101 (82.8%)		1[a]	
CT	13 (9.8%)	18 (14.8%)		1.62 (0.76−3.46)	0.213
TT	1 (0.8%)	3 (2.5%)		3.51 (0.36−34.22)	0.341
CT + TT[c]	14 (10.6%)	19 (15.6%)		1.59 (0.76−3.32)	0.219
Alleles			0.079		
C	249(94.3%)	220 (91.8%)		1[a]	
T	15 (5.7%)	24 (8.2%)		1.81 (0.93−3.54)	0.079
TIMP-2 −418 G/C			0.431		
GG	130 (98.5%)	118 (96.7%)		1[a]	
GC	2 (1.5%)	4 (3.3%)		2.22 (0.40−12.36)	0.431
CC	0 (0%)	0 (0%)		—	—
GC + CC[d]	2 (1.5%)	4 (3.3%)		2.22 (0.40−12.36)	0.431
Alleles			0.434		
G	262 (98.5%)	238 (98.3%)		1[a]	
C	2 (1.5%)	4 (1.7%)		2.19 (0.40−12.04)	0.434

CAD = coronary artery disease; CI = confidence interval; MI = myocardial infarction; MMP = matrix metalloproteinase; OR = ods ratio; TIMP = tissue inhibitors of metalloproteinase.
[a] Reference genotype/allele.
[b] Calculations were performed AA versus AG + GG.
[c] Calculations were performed CC versus CT + TT.
[d] Calculations were performed GG versus GC + CC.

the distribution of genotype and allele frequencies between CAD + MI and control groups for *TIMP-2* G-418C polymorphism. There are differences in terms of allele distribution of *MMP-7* or *TIMP-2* polymorphisms between the Turkish population and other racial populations. However, *MMP-7* A-181G and *TIMP-2* G-418C allele distributions, in particular, are similar to the those in the Caucasian population [24,25]. Other polymorphisms have not been mostly investigated.

When comparing CAD or MI groups to controls, AA, CC and GG genotypes were frequently observed in CAD or MI patients and controls for the *MMP-7* A-181G, C-153T and *TIMP-2* G-418C polymorphisms, respectively (Table 4; data not shown for CAD without MI). In cases and controls, the genotype and allele distributions of A-181G and G-418C polymorphisms were found to be similar. Thus, any significant association was monitored among the two groups in with regard to these polymorphisms ($p > 0.05$) (Table 4, data not shown for CAD without MI). Although the genotypes of *MMP-7* C-153T polymorphism had no appreciable discrepancy among MI and control groups ($p > 0.05$), the allele frequencies of C-153T polymorphism were significantly different between the two groups ($p < 0.05$) (Table 4). The frequency of −153T allele was less in controls as compared to MI patients (5.7% vs. 13.2%). Moreover, the OR for T allele was 2.52 (95% CI, 1.08−5.86; $p = 0.028$) when comparing to C allele (Table 4).

Table 5 Binary logistic regression model for CAD with or without MI.

Variables	*p*	OR (%95 CI)
MMP-153C/T	0.126	1.92 (0.83−4,44)
Sex (male)	**0.001**	2.87 (1.64−5,01)
Hypertension (+)	**0.003**	2.28 (1.32−3.93)
Family history (+)	**0.049**	1.79 (1.10−3.26)
Hypercholesterolemia(+)	**0.019**	1.91 (1.11−3.28)

CAD = coronary artery disease; CI = confidence interval; MI = myocardial infarction; MMP = matrix metalloproteinase; OR = odds ratio.
p-Values <0.05 are shown in bold.

When it comes to *MMP-7* C-115T polymorphism, because all cases and controls had the CC genotype, we found no statistically appreciable differences among cases and controls.

When the baseline characteristics of patients and *MMP-7* and *TIMP-2* genotypes were added as covariates, logistic regression revealed only sex (OR = 2.87), hypertension (OR = 2.28), family history (OR = 1.79), and hypercholesterolemia (OR = 1.91) to be the strongest determinants of CAD (Table 5). *MMP-7* and *TIMP-2* genotypes were not identified as risk factors for CAD according to binary logistic regression analysis. Moreover, the distribution of baseline

Table 4 Genotype and allele frequencies of MMP-7 and TIMP-2 polymorphsms for MI patients and controls.

Genotypes	Controls ($n = 132$)	Cases ($n = 38$)	*p*	OR (95% CI)	*p*
MMP-7 −181 A/G			0.993		
AA	48 (36.4%)	14 (36.8%)		1[a]	
AG	57 (43.2%)	16 (42.1%)		0.96 (0.43−2.17)	0.926
GG	27 (20,4%)	8 (21.1%)		1.02 (0.38−2.73)	0.975
AG + GG[b]	84 (63.6%)	24 (63.2%)		1.12 (0.67−1.85)	0.674
Alleles			0.957		
A	153 (58%)	44 (85.3%)		1[a]	
G	111 (42%)	32 (14.7%)		0.98 (0.46−2.07)	0.957
MMP-7 −153 C/T			0.096		
CC	118 (89.4%)	30 (78.9%)		1[a]	
CT	13 (9.8%)	6 (15.8%)		1.82 (0.64−5.17)	0.250
TT	1 (0.8%)	2 (5.3%)		7.87 (0.69−89.69)	0.114
CT + TT[c]	14 (10.6%)	8 (45.2%)		2.25 (0.86−5.85)	0.104
Alleles			**0.028**		
C	249 (94.3%)	66 (86.8%)		1[a]	
T	15 (5.7%)	10 (13.2%)		2.52 (1.08−5.86)	**0.028**
TIMP-2-418 G/C			0.179		
GG	130 (98.5%)	36 (94.7%)		1[a]	
GC	2 (1.5%)	2 (5.3%)		3.61 (0.49−26.53)	0.216
CC	0 (0%)	0 (0%)		—	—
GC + CC[d]	2 (1.5%)	2 (5.3%)		3.61 (0.49−26.53)	0.216
Alleles			0.217		
G	262 (98.5%)	74 (76.2%)		1[a]	
C	2 (1.5%)	2 (23.8%)		3.54 (0.49−25.56)	0.217

p values <0.05 are shown in bold.
CI = confidence interval; MMP = matrix metalloproteinase; OR = odds ratio; TIMP = tissue inhibitors of metalloproteinase.
[a] Reference genotype/allele.
[b] Calculations were performed AA versus AG + GG.
[c] Calculations were performed CC versus CT + TT.
[d] Calculations were performed GG versus GC + CC.

characteristics of all participants according to genotypes is shown in Table 6. We found that the CT and TT genotypes of *MMP-7* −153 C/T polymorphism were more common in the female group ($p < 0.05$). However, there was no significant difference in the CAD + MI group in this regard (data not shown).

MMP-7 haplotype distributions in the CAD + MI and control groups are shown in Table 7. In the control groups and CAD + MI, only four haplotype combinations were found. As expected, the most frequent haplotype was the −181A/−153C/−115C (ACC) haplotype both in patient and control groups. There were no statistically significant association among cases and controls in terms of all haplotypes.

Discussion

In several cardiovascular pathogenesis such as atherosclerosis and heart failure, alteration of MMP expressions in vascular and cardiac tissue have been suggested to occur in atherosclerosis, aneurysms, postangioplasty restenosis, and heart failure [18]. MMPs exist in the macrophages, smooth muscle cells, atherosclerotic plaque, and endothelial cells [9,10,26]. Of the MMPs, MMP-7 expression, for example, increases in macrophages and cardiomyocytes after MI [9,27]. MMPs contribute to left ventricular remodeling after MI [9]. In addition, mRNA and protein expression of *MMP-7* are increased in abdominal aortic aneurysms [10]. Lindsey and Zamilpa [15] have indicated that post-MI survival was improved in null MMP-7 mice. MMPs are endogenously inhibited by TIMPs, which have different biological responses. Increase of TIMP expression induces myofibroblast differentiation and cell proliferation of cardiac fibroblast. TIMP-2 protein levels show dual peaks at Weeks 2 and 16 after MI but did not change for the 1st week in rats [15].

In the promoter regions of *MMP* and *TIMP* genes, SNPs affect the *MMP* and *TIMP* expression at the transcription level [26]. There is growing evidence that functional SNP in matrix remodeling genes may play a prominent role for detecting the susceptibility to atherosclerosis and its clinical manifestations [10]. Various polymorphisms in the *MMP* family genes have been described and analyzed in different studies. *TIMP* polymorphisms have been also studied because the activity of MMPs is controlled by TIMPs, and the balance between MMPs and TIMPs is important in terms of vascular remodeling [4,28].

SNPs in the *MMP-7* gene might have been related to CAD, but the role of MMP-7 has not been entirely characterized in cardiovascular disease [18]. Two common polymorphisms (A-181G and C-153T) were described by Jormsjö et al [10] in the promoter region of *MMP-7* gene. According to the study of Jormsjö and colleagues [10], both polymorphisms affected the binding of nuclear proteins and transcriptional activity of the *MMP-7* gene in hypercholesterolemic patients with CAD. Therefore, the role of *MMP-7* in the matrix remodeling related to CAD was suggested by researchers. Two *MMP-7* polymorphisms have been shown to display allele-specific effects on the activity of its promoters [18]. In Jormsjö et al's [10] study, carriers of the −181G or −153T alleles had smaller reference luminal diameters prior to percutaneous transluminal coronary angioplasty (PTCA) in hypercholesterolemic patients. In addition, the −181G/

Table 6 Distribution of baseline characteristics of all participants according to genotype.

Characteristics	MMP-7 −181 A/G				MMP-7 −153 C/T				TIMP-2 −418 G/C			
	AA	AG	GG	*p*	CC	CT	TT	*p*	GG	GC	CC	*p*
Age (y)	60.1 ± 11.2	57.88 ± 10.8	59.7 ± 11.4	0.328	58.6 ± 11.2	61.22 ± 9.3	63 ± 13.9	0.919	59.02 ± 11.2	57.66 ± 7.3	—	0.854
Sex												
Male/female	45/40	70/48	23/28	0.223	126/93	11/20	**1/3**	**0.035**	135/113	3/3	—	1.000
Smoking habits												
Smokers/nonsmokers	38/47	49/69	23/28	0.866	99/120	10/21	1/3	0.300	106/142	4/2	—	0.408
Hypercholesterolemia												
With/without	39/46	63/55	27/24	0.540	107/112	20/11	2/2	0.264	126/122	3/3	—	1.000
Hypertension												
With/without	45/40	61/57	28/23	0.928	114/105	19/12	1/3	0.335	131/117	3/3	—	1.000
Diabetes mellitus												
With/without	22/63	30/88	12/39	0.951	52/167	11/20	1/3	0.371	63/185	1/5	—	1.000
Family history												
With/without	25/60	34/84	16/35	0.945	67/152	7/24	1/3	0.645	74/174	1/5	—	0.673

p values <0.05 and <0.001 are shown in bold.
MMP = matrix metalloproteinase; TIMP = tissue inhibitors of metalloproteinase.

Table 7 MMP-7 haplotype distributions and their association with CAD + MI.

Haplotype	Controls		Cases		OR (95% CI)	*p*
	Frequency	$2n = 264$	Frequency	$2n = 244$		
ACC	0.5744	152	0.5293	129	1[a]	
GCC	0.3688	98	0.3723	91	1.09 (0.76–1.58)	0.633
GTC	0.0517	13	0.0744	18	1.63 (0.78–3.46)	0.198
ATC	0.0052	1	0.024	6	7.07 (0.84–59.4)	0.054

CAD = coronary artery disease; CI = confidence interval; MI = myocardial infarction; MMP = matrix metalloproteinase; OR = odds ratio.
[a] Reference haplotype.

−153T haplotype was found to be quite limited in a hypercholesterolemic patient with the smallest luminal diameter (2.50 mm prior to PTCA), and this allelic combination is associated with the highest transcription activity *in vitro*. However, analyses involving much larger cohorts or further studies have to be performed to elucidate these effects. In our study, the most frequent haplotype was the −181A/−153C/−115C (ACC) haplotype. We did not find any significant relation between cases and control with regard to all haplotypes. In light of these results, various studies about *MMP-7* polymorphisms have been studied in different diseases and diverse populations. Panayiotou et al [29] indicated that *MMP-7* −181A allele was related with the presence of atherosclerotic plaque in carotid and femoral arteries in middle-aged men. In two earlier studies, no significant association with *MMP-7* A-181G and left ventricular dysfunction (LVD) was found in a North Indian population [3,30]. Similarly, we did not find any significant association of susceptibilty to CAD with or without MI and *MMP-7* polymorphisms including A-181G and C-115T. When it comes to *MMP-7* C-115T polymorphism, there were no statistically significant discrepancy between cases and controls because only the CC genotype was found in all groups. However, although no significant discrepancies among CAD and controls was not observed in terms of the genotype distribution of the C-153T polymorphism, allele frequencies of *MMP-7* C-153T polymorphism were appreciable different between the MI group and controls. Therefore, our results suggested that each T-allele of C-153T polymorphism can be a risk for MI development in CAD patients with an OR of 2.52. Because of the elevation of *MMP-7* expression in macrophages and cardiomyocytes after MI [9], −153T allele could have increased transcriptional activity and can be a risk for MI development. However, further investigation in a large chort is necessary to clarify the association of *MMP-7* C-153T polymorphism with MI development in CAD patients because of the small sample size used in this study.

Previously described SNP, named *TIMP-2* G-418C, is located in the consensus sequence of an Sp1 binding region [17]. Several epidemiological analyses have studied the relationship between *TIMP-2* G-418C polymorphism and various diseases such as hypertension in patients with MI, chronic obstructive pulmonary disease (COPD), periodontitis, and different malignancies, because this variation may downregulate *TIMP-2* expression [14,16,21,31]. However, association between CAD and G-418C polymorphism has been poorly studied until now as we look at the literature. In our study, there were no appreciable discrepancies in the genotype distribution and allele frequencies of *TIMP-2* G-418C polymorphism between CAD + MI and CAD or MI patients and controls. Thus, we did not show the relation of this polymorphism on CAD or MI because of the high frequency of the GG genotype in cases and control. Similar to our study, the frequency of *TIMP-2* G-418C polymorphism appears to be very low among Caucasian individuals of the Southeastern region of Brazil [14]. However, in a Japanese population, this polymorphism was related with the pathogenesis of COPD [16]. By contrast, *TIMP-2* G-418C polymorphism did not contribute toward the development of COPD in the Egyptian population [17]. Other studies have noticed that *TIMP-2* G-418C polymorphism is susceptible to breast and gastric cancer, varicose vein, hypertensive heart disease, and the magnitude of QT and QTc dispersion prolongation in an elderly Chinese population [12,21,28,32,33]. In addition, Mikołajczyk-Stecyna et al [34] have indicated that this polymorphism is an independent risk factor of abdominal aortic aneurysm in individuals in a Polish population.

Conclusion

This study is the first report to clarify the appreciable relationship between *MMP-7* C-153T polymorphism and MI development in CAD patients. The other polymorphisms did not show any significant discrepancy between cases and controls in all groups. We would like to emphasize that the main limitation of our study is the sample size owing to the exclusion of patients with mild CAD (<50% stenosis in one or more epicardial vessels). In addition, several participants were also excluded because of the abnormal clinical characteristics observed in the control group. Therefore, further investigation in a large chort is necessary to clarify the both association of *MMP-7* C-153T polymorphism with MI development in CAD patients and *MMP-7* and *TIMP-2* polymorphisms contributing to the development of CAD or MI. These findings also need further confirmation in other populations. Besides these polymorphisms, further studies of different polymorphisms in *MMP-7* or *TIMP-2* gene and other MMP family members or *TIMP* genes would be beneficial to shed light on the relationship between CAD and *MMPs* or *TIMP* gene polymorphisms.

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