



## rs3918242 *MMP9* gene polymorphism is associated with myocardial infarction in Mexican patients

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**ABSTRACT.** Several studies have demonstrated that matrix metalloproteinases (MMPs) play a major role in atherosclerotic plaque disruption and lead to myocardial infarction (MI). We investigated the association between the *MMP1* -1607 1G/2G (rs1799750), *MMP3* -1612 5A/6A (rs3025058), and *MMP9* -1562 C/T (rs3918242) polymorphisms and the risk of developing MI in a Mexican mestizo cohort. The genotype analysis was performed using the restriction fragment length polymorphism-polymerase chain reaction technique in a group of 236 patients with a history of MI and 285 healthy controls. Similar distributions of rs1799750 and rs3025058 were observed in both groups; however,

the *MMP9* rs3918242 *T* allele and the *CT* genotype were associated with the risk of developing MI (OR = 2.32, *p*C = 0.02 and OR = 2.40, *p*C = 0.02, respectively). Multiple logistic analysis was performed between MI patients and controls to estimate the risk, and after adjusting for identified risk factors, the *CT* + *TT* genotypes of *MMP9* rs3918242 were found to be significantly associated with increased risk of developing MI than those with the *CC* genotype (OR = 2.88, *P* < 0.01). In summary, our results reveal that the rs3918242 polymorphism of the *MMP9* gene plays a major role in the risk of developing MI.

**Key words:** Matrix metalloproteinase; Polymorphisms; Myocardial infarction; Atherothrombosis

## INTRODUCTION

Globally, coronary artery disease (CAD) is one of the main causes of mortality, and myocardial infarction (MI) is one of the main clinical presentations of its pathology. MI is a complex disease involving multiple genetic and environmental factors. The main environmental factors underlying atherosclerosis and CAD development include smoking, diabetes, hypertension, obesity, and hypercholesterolemia. Recently, numerous studies have provided evidence that genetic polymorphisms play a pivotal role in the development of CAD and MI (Wang et al., 2015).

Current evidence indicates that disturbances in arterial extracellular matrix remodeling play an important role in the atherosclerotic process. The activity of metalloproteinases (MMPs) may contribute to the pathophysiology of atherosclerosis by facilitating the migration of vascular smooth muscle cells into the internal elastic lamina at the intima of the vessel wall, where they proliferate and contribute to plaque establishment and growth (Herzog et al., 1998; Newby, 2005). The MMPs are a family of zinc-dependent endopeptidases responsible for degrading extracellular components (Creemers et al., 2001; Falk, 2006). Each MMP has its own substrate specificity (Ratnikov et al., 2014). Previous studies have suggested that MMPs may play a role in the development of several diseases, such as spontaneous early pregnancy failure (Nissi et al., 2013), solid and hematological malignancies (Chaudhary et al., 2013), and abdominal aortic aneurysms (Duellman et al., 2012). Several polymorphisms in the genes encoding MMP molecules have been studied in cardiovascular diseases (Chehaibi et al., 2014; Olsen et al., 2014; Opstad et al., 2014). It is essential to note that association study results may differ among populations owing to genetic variability between populations, including differences in allele frequencies and linkage disequilibrium structure (Neale and Sham, 2004). The variation in CAD risk between individuals is heritable, indicating that genetic risk factors play a critical role in pathogenesis.

The Mexican population is highly heterogenic. Nevertheless, 98% of the population are Mestizos, the descendants of the native Amerindians and immigrants from Europe and Africa who arrived in Mexican territory more than 500 years ago, during the period when Mexico was a Spanish colony. The remaining 2% of the Mexican population comprises other groups, mainly Amerindians (Camacho-Mejorado et al., 2015).

In the current study, we included the coronary calcium scores of the study population in an attempt to exclude controls with subclinical atherosclerosis. This factor has been the main strength of other reports. Therefore, the aim of the present study was to be the first study to determine if there is a significant association between *MMP1*, *MMP3*, and *MMP9* gene polymorphisms and

MI in the Mexican Mestizo population. We also investigated the relationship between *MMP* gene polymorphisms and cardiovascular risk factors.

## MATERIAL AND METHODS

### Subjects

A letter of consent was obtained from all participants. The study complies with the Declaration of Helsinki and was approved by the Ethics and Research Committee of Instituto Nacional de Cardiología "Ignacio Chávez". This study included 236 Mexican Mestizo patients with a history of MI (194 men and 42 women). MI was clinically characterized by classic angina symptoms and defined as an elevation of 1 mm or more of the ST-segment, as well as the development of a Q wave in two or more contiguous electrocardiographic records, and/or thrice the upper limit of serum creatine phosphokinase MB isoenzyme (normal value = 0.6-6.3 ng/mL) in at least one blood sample.

A group of 285 healthy unrelated individuals (134 men and 151 women) who did not have any history compatible with stable angina or a previous diagnosis of systemic or cardiovascular disease (corroborated clinically and by electrocardiography) was used as a control group. The coronary calcium score was determined by multidetector computed tomography to identify subclinical atherosclerosis; all control individuals were required to have a value of zero. Anthropometric measures were recorded and medical history information was collected on traditional risk factors in all included participants. We used clinical criteria to identify type 2 diabetes mellitus patients: a serum glucose level of 126 mg/dL or higher in two samples, a previous diagnosis, or current hypoglycemic treatment and/or insulin. Hypertensive patients were identified by a previous diagnosis or by participation in active antihypertensive therapy. Dyslipidemia was defined as total cholesterol of 200 mg/dL or higher, low-density lipoprotein cholesterol  $\geq$ 130 mg/dL, or serum triglycerides of 150 mg/dL or higher. Active smokers were identified by a consumption habit of five or more tobacco cigarettes per day, or abstinence for less than twelve months.

Individuals were considered to be active alcohol drinkers if they drank more than 6 g daily. Body mass index was calculated by a standard formula (weight in kg/squared height). Coronary angiography was performed in all patients. The extent of CAD was estimated by determination of the number of stenotic coronary arteries, classified as one-, two-, or three-vessel disease. Involvement of two vessels meant significant stenosis in two coronary vessels or a narrowing of  $\geq$ 50% in the left main trunk coronary artery, and involvement of three vessels meant significant stenosis in three coronary vessels. Involvement of one vessel was considered hemodynamically significant with more than a 50% reduction in the diameter of an important vessel (right coronary, left anterior descending, or circumflex).

For this study, a Mexican Mestizo was defined as a person born in Mexico who was the descendant of the original autochthonous inhabitants of the region and of individuals, mainly Spaniards, of Caucasian and/or African origin, who came to America during the colonization period. Therefore, all subjects included were ethnically matched, and were considered to be Mexican Mestizo only if they were at least third-generation Mexicans.

### DNA extraction

The Lahiri and Nurnberger method was used to isolate genomic DNA from blood cells (Lahiri and Nurnberger, 1991).

## Polymorphism analysis (*MMP1*, *MMP3*, and *MMP9*)

*MMP1* -1607 1G/2G (rs1799750), *MMP3* -1612 5A/6A (rs3025058), and *MMP9* -1562 C/T (rs3918242) polymorphisms were determined using the restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) technique (Table 1).

## Statistical analysis

The allele and genotype frequencies of the polymorphisms were obtained by direct counting. The Hardy-Weinberg equilibrium (HWE) was calculated using the chi-square test. Statistical analysis was carried out with SPSS for Windows. Comparison of numerical variables between the groups was performed using the Mann-Whitney U-test; data are reported as medians and 25 and 75 percentiles. Categorical variables were analyzed using the chi-squared or Fisher test as required, and are reported as absolute frequencies and proportions. Statistical significance was set at  $P < 0.05$ . Multiple logistic analysis was used to test for association of polymorphisms with MI adjusted for gender, smoking habits, diabetes mellitus, and hypertension. All  $P$  values quoted were corrected ( $p_C$ ) by the Bonferroni test for multiple comparisons.

## RESULTS

### Sample characteristics

The demographic and clinical characteristics of the study groups are presented in Table 2. As expected, type 2 diabetes mellitus, hypertension, and smoking habits were more frequent in MI patients than in healthy controls. In the patient group, 6.3% showed no occluded vessels, 32.6% exhibited a one-vessel occlusion, 30.1% a two-vessel occlusion, and 30.9% a three-vessel occlusion. Moreover, 9.7% of patients presented with coronary calcification.

### Allele and genotype frequencies

The allele and genotype frequencies of *MMP1* -1607 1G/2G (rs1799750), *MMP3* -1612 5A/6A (rs3025058), and *MMP9* -1562 C/T (rs3918242) gene polymorphisms in healthy controls and MI patients are shown in Table 3. The observed and expected frequencies of all polymorphic sites were in HWE. The distribution of *MMP1* -1607 2G/1G (rs1799750) and *MMP3* -1612 5A/6A (rs3025058) was similar in both groups; however, there was a difference in the *MMP9* -1562 C/T (rs3918242) gene polymorphism distribution between the MI patients and the healthy controls. Patients showed increased frequencies of the *T* allele and the *CT* genotype compared with the healthy controls (OR = 2.32; 95% CI = 1.15-4.73;  $p_C$  = 0.02 and OR = 2.40; 95% CI = 1.17-4.97;  $p_C$  = 0.02, respectively). However, the *MMP9* -1562 C/T (rs3918242) *C* allele and *CC* genotype frequencies decreased in MI patients compared with the healthy controls (OR = 0.43; 95% CI = 0.21-0.87;  $p_C$  = 0.02 and OR = 0.42; 95% CI = 0.20-0.86;  $p_C$  = 0.02, respectively). Multiple logistic analysis was used to estimate the risk between the MI patients and the controls adjusted by gender, smoking habits, diabetes mellitus, and hypertension. In this analysis, the *MMP9* -1562 (rs3918242) *CT* + *TT* genotypes were significantly associated with the risk of developing MI compared with the *CC* genotype (OR = 2.88; 95% CI = 1.18-6.99;  $P < 0.01$ ) (data not shown).

**Table 1.** Primer sequences for MMP1, MMP3, and MMP9 polymorphisms.

Gene	Polymorphisms	Forward primer (5'-3')	Reverse primer (5'-3')	Enzyme	Size	References
MMP1	-1607 1G/2G (rs1799750)	TGACTTTTAAACATAGTCTATG TTCA	TC TTGGATTGATTTGAGATAA GTCATAGC	<i>AclI</i>	1G (241 + 28 bp) 2G (269 bp)	Fang et al., 2005
MMP3	-1612 5A/6A (rs3025058)	GGTTCCTCCATTCCCTTTGATGGG GGGAAAGA	CTTCCTGGAATTTACATCAC TGCCACCACT	<i>PsiI</i> (7#111)	1G/2G (269+241+28 bp) 5A (97+32 bp) 6A (129 bp) 5A/6A (129+97+32 bp)	Fang et al., 2005
MMP9	-1562 C/T (rs3918242)	GCCTGGCACATAGTAGGCCC	CTTCCTAGCCAGCCGGCATC	<i>PaeI</i> (SphI)	CC (435 bp) TT (188 bp) CT (435+247+188 bp)	Medley et al., 2004.

**Table 2.** Baseline clinical data of the study groups.

Variable	Control group (N = 285)			MI patients (N = 236)			P value
	P25	Median	P75	P25	Median	P75	
Age (years)	55	58	63	52	59	66	NS
Gender [N (%)]							
Male		134 (47%)			194 (82%)		< 0.001
Female		151 (53%)			42 (17.8%)		
BMI (kg/m <sup>2</sup> )	26	28	30.5	25	27	29	0.002
Obese (%)							
Yes		71 (24.9%)			37 (15.6%)		0.014
No		214 (75.1%)			199 (84.4%)		
Type 2 diabetes mellitus (%)							
Yes		37 (13%)			102 (43.2%)		< 0.001
No		248 (87%)			134 (56.8%)		
Hypertension (%)							
Yes		145 (50.9%)			171 (72.5%)		< 0.001
No		140 (49.1%)			65 (27.5%)		
Dyslipidemia (%)							
Yes		197 (69.1%)			150 (63.6%)		NS
No		88 (30.9%)			86 (36.4%)		
Smoking habits (%)							
Yes		47 (16.5%)			160 (67.8%)		< 0.001
No		238 (83.5%)			76 (32.2%)		
Use of alcohol (%)							
Yes		144 (50.5%)			85 (36%)		0.001
No		141 (49.5%)			151 (64%)		
Coronary angiography							
No occluded vessels					14 (6.3%)		
One vessel					77 (32.6%)		
Two vessels					72 (30.1%)		
Three vessels					73 (30.9%)		
Coronary calcification							
Yes					23 (9.7%)		
No					213 (90.3%)		

MI = myocardial infarction; BMI = body mass index.

**Table 3.** Allele and genotype frequencies (af and gf, respectively) of *MMP1* -1607, *MMP3* -1612, and *MMP9* -1562 polymorphisms in myocardial infarction (MI) patients and healthy controls.

Gene SNP	Genotype	Controls (N = 285)		MI (N = 236)		pC	OR (95% CI)
		N	gf	N	gf		
<i>MMP1</i> -1607 (rs1799750)	1G1G	1	0.003	2	0.008	NS	
	1G2G	38	0.133	34	0.144		
	2G2G	246	0.863	200	0.847	NS	
	Allele	N	af	N	af		
	1G	40	0.070	38	0.080		
<i>MMP3</i> -1612 (rs3025058)	2G	530	0.929	434	0.919	NS	
	Genotype	N	gf	N	gf		
	6A6A	141	0.494	114	0.483	NS	
	6A5A	121	0.424	105	0.444		
	5A5A	23	0.080	17	0.072		
Allele	N	af	N	af			
	6A	403	0.707	333	0.705		
<i>MMP9</i> -1562 (rs3918242)	5A	167	0.292	139	0.294	NS	
	Genotype	N	gf	N	gf		
	CC	271	0.950	210	0.889	<b>0.02</b>	<b>0.42 (0.20-0.86)</b>
	CT	14	0.049	26	0.110		
	TT	0	0.000	0	0.000	<b>0.02</b>	<b>2.40 (1.17-4.97)</b>
	Allele	N	gf	N	af		
	C	556	0.975	446	0.944		<b>0.43 (0.21-0.87)</b>
	T	14	0.024	26	0.055	<b>0.02</b>	<b>2.32 (1.15-4.73)</b>

MI = myocardial infarction; SNP = single nucleotide polymorphism; rs = reference SNP; OR = odds ratio; 95% CI = 95% confidence interval; pC = P-corrected; NS = not significant. Bold numbers indicate significant associations.

## DISCUSSION

The pathophysiology of MI includes the transition of a stable coronary atherosclerotic lesion onto a ruptured plaque. After the rupture, a thrombus is formed, which is conducive to a hypoxic lesion and is clinically manifested as MI. MMPs, mainly synthesized by the infiltrating cells, degrade the extracellular matrix and contribute to the weakening of the fibrous cap and its subsequent rupture (Brown et al., 1995). It is noteworthy that polymorphisms in *MMP* genes have been studied in several diseases with contradictory results (Alp et al., 2009; Zhi et al., 2010; Alp et al., 2011). Association of these polymorphisms has been observed in CAD (Opstad et al., 2013), and intracerebral hemorrhage (Yang et al., 2014). In the present study, we analyzed three *MMP* gene polymorphisms in Mexican Mestizo patients with MI. The distribution of the *MMP1* -1607 1G/2G (rs1799750) and *MMP3* -1612 5A/6A (rs3025058) polymorphisms was similar in both groups. However, we found a different distribution of the -1562 (rs3918242) polymorphism of the *MMP9* gene compared with the healthy controls. The increased frequencies of the *T* allele and *CT* genotype of this polymorphism supports its role as a genetic susceptibility marker for MI in the studied population. In fact, increased levels of *MMP9* in the atherosclerotic plaques of coronary arteries have been reported (Pasterkamp et al., 2000).

The polymorphic sites were selected based on previous association studies in cardiovascular diseases and their important role at the functional level. The *MMP1* 1G and 2G alleles differ in the insertion of one guanine nucleotide. The polymorphism site is located at position -1607 relative to the transcriptional start site of the gene. The transcriptional activity of the 2G allele is 20 times stronger than that of 1G. Hence, carriers of the former allele will overexpress *MMP1*, which can lead to a diminished collection of collagen. This is known to induce atherosclerosis (Ye, 2000).

The 5A and 6A alleles of the *MMP3* gene differ in a single adenine insertion and include either five or six adenine nucleotides at position -1612 of the promoter region. Both homozygous and heterozygous carriers of the 5A allele have a higher risk of CAD than 6A carriers, since the transcriptional activity of the 5A allele is twice as active. (Liu et al., 2003; Liu et al., 2005).

*MMP9* polymorphisms are caused by a single C/T base change at -1562 (the promoter region). Unlike the *C* allele, the *T* allele is associated with CAD, since it enhances gene expression (Ye, 2000).

Román-García et al. (2009) evaluated three *MMP1* polymorphisms (rs11292517, rs1144393, and rs514921) in 261 male patients with MI. They found no association between MI and the *MMP1* polymorphisms. These results are in agreement with our study, since we also found that the same gene (albeit a different variant) was not associated with the disease.

In another report, Sakowicz et al. (2013) analyzed 17 polymorphisms in 15 candidate genes. Among them, *MMP3* -1612 5A/6A (rs3025058) and *MMP9* -1562 (rs3918242) gene polymorphisms were examined in a cohort of 271 patients with MI and 141 healthy controls in a white Caucasian population. They reported that the *MMP3* -1612 5A/6A (rs3025058) polymorphism was associated with advanced stenosis in arterial vessels, whereas the *MMP9* -1562 (rs3918242) *T* allele was associated with hypertension and a higher risk of MI. In our study, we did not detect an association between the -1612 5A/6A (rs3025058) polymorphism of the *MMP3* gene and the disease. Sakowicz et al. (2013)'s findings on the *MMP9* -1562 (rs3918242) polymorphism were in line with our results; when a logistic regression analysis was performed to determine the association between cardiovascular risk factors and the *CT* + *TT* genotypes of this polymorphic site, we found an association only with *MMP9* -1562 (rs3918242).

In addition, Qintao et al. (2014) reported a case-control study in which they examined

nine polymorphisms of the *MMP1* gene in 438 healthy controls and 411 patients with CAD from a Chinese Han population. Their analysis revealed a weak association between the rs1799750 polymorphism and CAD, while we found no such association with the same polymorphism. This discrepancy may be related to differences in genetic background in the study population or in the selection of patients and healthy controls. Furthermore, Sewelam et al. (2013) studied the association between the *MMP3* and *MMP9* gene polymorphisms and MI in an Egyptian population and reported an association of the *MMP3* -1612 5A/6A (rs3025058) and *MMP9* -1562 (rs3918242) *CT* + *TT* genotypes and the risk of developing MI. In our study, the same polymorphic sites were analyzed. However, we only detected an association in the *CT* + *TT* genotypes of the *MMP9* -1562 (rs3918242) polymorphism. The principal differences between these studies were the number of patients included and the number of polymorphisms analyzed. In their report, Sewelam et al. (2013) included only 40 patients compared with the 236 patients analyzed in our study. We examined three polymorphisms in different *MMP* genes, whereas Sewelam et al. (2013) analyzed only two polymorphisms. The association between *MMP1* -1607 1G/2G (rs1799750) and *MMP3* -1612 5A/6A (rs3025058) polymorphisms and MI was not corroborated in a large Caucasian population study, and only the *MMP9* -1562 (rs3918242) polymorphism was associated with MI (Horne et al., 2007). This is congruent with our results. Recently, Nie et al. (2014) found that the *MMP9* -1562 (rs3918242) *T* allele was associated with a 1.5-fold increase in ischemic stroke risk in the Chinese population. Importantly, this finding is in line with our results.

The main strength of this study is the use of a coronary calcium score via computed tomography. This is a specific method to evaluate the coronary calcium level, and was, therefore, used to diagnose subclinical atherosclerosis. Indeed, the control individuals were selected if they had a coronary artery calcium (CAC) level of zero (CAC = 0). We only selected patients where the MI etiology presented was atherosclerotic plaque disruption. The reason for this was that the relevance of MMPs lies in the degradation of the extracellular matrix of the coronary plaque, including the thinning of the fibrous cap. This study strength must be considered when comparing our findings with similar studies.

However, some study limitations need to be addressed. Here, we only included the analysis of one polymorphism of each gene. Also, the findings were based on a limited sample size. We therefore suggest exercising caution when interpreting them.

In summary, our data suggest an association between a functional polymorphism in the promoter region of the *MMP9* gene and the risk of developing MI in the Mexican Mestizo population. Although this is the largest study carried out in this population, our data are preliminary owing to the sample size. Consequently, additional studies in larger populations will be required to define the true genetic role of these polymorphisms in MI.

## Conflicts of interest

The authors declare no conflict of interest.

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