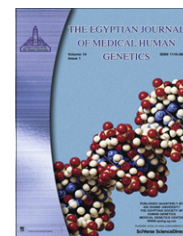




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ORIGINAL ARTICLE

# Association between the polymorphisms of matrix metalloproteinases 9 and 3 genes and risk of myocardial infarction in Egyptian patients

Nadia I Sewelam <sup>a,\*</sup>, Eman R. Radwan <sup>a</sup>, Ashraf W Andraos <sup>b</sup>,  
Baher E Ibrahim <sup>c</sup>, Manal M. Wilson <sup>a</sup>

<sup>a</sup> Department of Clinical and Chemical Pathology, Cairo University, Egypt

<sup>b</sup> Faculty of Medicine, Cairo University, Egypt

<sup>c</sup> Department of Clinical and Chemical Pathology, National heart institute, Egypt

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## KEYWORDS

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**Abstract** The present study investigated the relationship between the genetic polymorphisms in MMP-9 and MMP-3 genes and acute myocardial infarction (AMI). We examined 40 patients with acute myocardial infarction and 40 age and sex matched controls for MMP-9 functional promoter polymorphism (–1562 C > T) and MMP-3 (5A/6A) deletion/insertion polymorphism using restriction fragment length polymorphism (RFLP) for amplified genomic DNA. The frequencies of the combined mutant genotypes CT and TT in the (–1562 C > T) MMP9 were significantly higher in AMI patients (20%) when compared to the controls (0%) ( $p$  value = 0.005) showing an association between these genotypes and AMI. Also there was a significant difference between 5A/5A genotype and 5A allele frequencies when both are compared in the patients (25% and 35%) and the controls (2.5% and 18.75%) ( $p$  = 0.009; OR = 13; CI = 1.576–107.233); and ( $p$  = 0.02; OR = 2.333, CI = 1.130–4.820) respectively. In conclusion, the –1562C > T polymorphism of the MMP9 gene is strongly associated with acute myocardial infarction in the Egyptian population. Furthermore, our study supported the presence of the 5A/5A genotype of MMP3 gene promoter polymorphism as a risk factor of AMI in Egyptian patients. Meanwhile, the race selection should be paid more attention since the pathogenesis of a disease might have different bases in different racial population groups.

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\* Corresponding author. Address: 50, Mekias Street, Manial El Rouda, Cairo 11451, Egypt. Tel.: +20 2 25327392/1 284979979.

E-mail addresses: nadiasw@hotmail.com (N.I Sewelam), emanradwan@gmail.com (E.R. Radwan), mmmw@tedata.net.com (M.M. Wilson).

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## 1. Introduction

Myocardial infarction is caused by several inherited and acquired risk factors that predispose to the development of atherosclerotic lesions and plaque rupture. This rupture of an atherosclerotic plaque is a major event in the pathogenesis of an acute myocardial infarction (AMI) [1]. Plaques that are

vulnerable to rupture tend to have a large lipid rich atheromatous core, a thin fibrous cap covering the core, active inflammatory cellular infiltration, particularly in the plaque shoulders affected by strong shear stress, and reduced smooth muscle cell density collagen content in the fibrous cap. Matrix metalloproteinases (MMPs), mainly produced by infiltrating cells, degrade the extracellular matrix, thereby contributing to the weakening of the cap and its subsequent rupture [2]. MMPs are a family of more than 20 zinc-dependent proteinases with proteolytic activity against extracellular matrix (ECM) components including collagen and elastin, as well as numerous proteins involved in angiogenesis, cell migration, growth and apoptosis [3]. Each MMP has distinct although overlapping substrate specificities. Previous studies have suggested that MMPs may play a role in cardiovascular and cerebrovascular diseases [4].

Of the many matrix metalloproteinases, MMP-9 (gelatinase B) might play an important role in matrix degradation and the subsequent rupture of the atherosclerotic plaques, owing to its broad substrate specificity distal position in the matrix proteolytic cascade. MMP-9 is involved in the degradation of a broad spectrum of substrates, including collagen types IV, V, VII and X as well as gelatin, and also degrades the proteoglycans and the elastins [5]. A functional single nucleotide polymorphism (SNP) in the MMP-9 promoter, with a cytosine to thymidine transition at position -1562, was found. According to in vitro assays of the promoter activity, the T allele had a higher promoter activity than the C allele [6], and was associated with an elevation in both the levels of MMP-9 expression level and serum MMP-9 in humans. An association of a SNP in the MMP-9 promoter with coronary artery disease and risk of myocardial infarction lesions has also been reported [7].

Stromelysin is a metalloproteinase (MMP-3) involved in the turnover of the extracellular matrix components. This enzyme exhibits a wide spectrum of activity: it is able to degrade proteoglycans, collagens III, IV, V, and IX, laminin, fibronectin, gelatin, and elastin. MMP-3 has been found to be extensively expressed in atherosclerotic plaques by macrophage origin and smooth muscle cells in the plaque cap, intima, and adventitia [8]. This finding has led to the hypothesis that MMP-3 might be involved in plaque rupture, followed by platelet activation and initiation of the coagulation cascade [9]. The promoter of human MMP3 contains a common deletion/insertion polymorphism, characterized by runs of 5 or 6 adenosines, 1612 bp upstream from the start site of transcription (rs3025058, also known as 5A/6A polymorphism) [10]. The aim of the present study was to investigate whether polymorphisms in MMP-9 and MMP-3 genes are a significant risk factor for acute myocardial infarction in an Egyptian Cohort.

## 2. Subjects and method

### 2.1. Study design

Case-control study both descriptive and analytic.

### 2.2. Subjects

The study was carried out in accordance with the Helsinki Declaration. The study was approved by the ethics committee of Faculty of Medicine, Cairo University.

This study was conducted on 40 unrelated Egyptian patients presenting with acute myocardial infarction (AMI) and 40 healthy Egyptian control subjects age and sex matched. All patients were recruited from the Intensive Care Unit Department, Cairo University Hospital during the period from April 2012 to October 2012. Consents were taken from all participants before being involved in the study.

Patients were diagnosed as having AMI according to the World Health Organization criteria and the Consensus Document of the Joint European Society of Cardiology/American College of Cardiology Committee for the Redefinition of a Myocardial Infarction [11].

### 2.3. Method

#### 2.3.1. Patients were subjected to the following

- (1) History taking and clinical assessment.
- (2) Twelve lead electrocardiogram (ECG) to confirm the diagnosis of ST elevation in MI.
- (3) Laboratory investigations including; CK, CK-MB, LDH, Troponin, AST, ALT and creatinine.
- (4) Polymerase chain reaction, restriction fragment length polymorphism (RFLP) for the detection of MMP9 -1562C > T and MMP3 5A/6A polymorphisms.

#### 2.3.2. DNA extraction

Genomic DNA was isolated from 2 ml venous blood sample withdrawn on EDTA using genomic DNA purification kit (*Fermentas*) according to the manufacturer's instructions.

#### 2.3.3. Genotyping

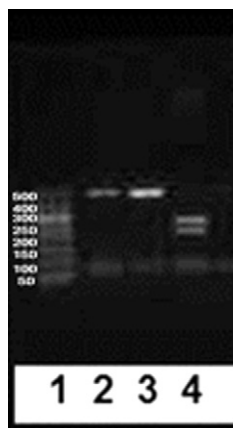
Gene polymorphisms were detected through PCR amplification followed by digestion using restriction endonuclease enzymes for RFLP analysis. The genotyping of MMP-9 promoter at position -1562 was detected by using the forward primer, (5'-GCC TGG CAC ATA GTA GGC CC-3') corresponding to segment from base pair number (-1871 to -1851) and the reverse primer, (5'-CTT CCT AGC CAG CCG GCA TC-3') corresponding to segment from base pair number (-1339 to -1319), as previously described [12]. MMP3 gene 5A/6A polymorphism was genotyped using the forward primer (5'-GGT TCT CCA TTC CTT TGA TGG GGG GAA AgA-3'); and the reverse primer (5'-CTT CCT GGA ATT CAC ATC ACT GCC ACC ACT-3'), as previously described [13]. The second nucleotide A in the forward primer 3' end was substituted by G in order to facilitate change in the 5A allele and generate the recognition sites for *Psy I* (*Tth111 I*) (GACN/NNGTC).

PCR reaction for both polymorphisms was performed in a 25 µl volume with 5 µl DNA (50 ng), 1 µl forward primer and 1 µl reverse primer (the primer concentration was 20 pmol for each primer), 12.5 µl master mix (*Dreamtaq Green PCR, Fermentas*) and 5.5 µl nuclease free water. Amplification was performed on an automated thermal cycler (*Applied Biosystems, USA*). PCR conditions for MMP 9 polymorphism were; 5 min for initial denaturation at 94 °C; 35 cycles at 94 °C for 15 s for denaturation, 30 s at 62 °C for annealing and 30 s at 72 °C for extension, followed by 8 min at 72 °C for final extension [14]. PCR conditions for MMP 3 polymorphism were;

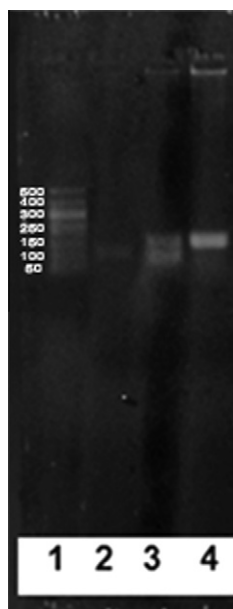
5 min at 95 °C for initial denaturation; 30 cycles of denaturing for 30 s at 94 °C, 30 s annealing at 65 °C, and 1 min extension at 72 °C, followed by final extension at 72 °C for 10 min [13].

#### 2.3.4. Restriction enzyme digestion

**2.3.4.1. MMP-9.** The PCR products were digested with SphI restriction endonuclease (*Fast Digest, Thermo Scientific*) and subjected to electrophoresis on a 2% agarose gel and the bands were visualized by ethidium bromide staining under U/V light. The PCR product of -1562 C allele is not cleaved by SphI, while that of the -1562 T allele is cleaved by the enzyme generating 247 and 188 bp fragments [15] (Fig. 1).



**Figure 1** PCR-RFLP analysis of -1562C>T MMP-9 polymorphism in our study. Lane 1: 50 bp DNA molecular weight marker (Qiagen, USA), Lanes 2 and 3: show CC genotype (435). Lane 4: shows TT genotype (247 and 188 bp).



**Figure 2** PCR-RFLP analysis of 5A/6A MMP-3 polymorphism in our study. Lane 1: 50 bp DNA molecular weight marker (Qiagen, USA). Lane 2: shows 5A/5A genotype (97 bp). Lane 3: shows 5A/6A genotype (130 and 97 bps). Lane 4: shows 6A/6A genotype (130 bps).

**2.3.4.2. MMP-3.** The PCR product was digested with Psy I (*FastDigest, Thermo Scientific*) The 6A/6A genotype has no restriction sites, producing therefore one 130 bp band after digestion; in the 5A/6A genotype only the 5A allele is cut, showing three bands: 130 bp, 97 bp, 33 bp; in 5A/5A genotype both alleles are cut into two bands: 97 bp and 33 bp, although only the 97 bp can be seen on the gel [13] (Fig. 2).

#### 2.4. Statistical methods

Data were statistically described in terms of mean  $\pm$  standard deviation ( $\pm$ SD), range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using the Mann Whitney U test for independent samples when comparing 2 groups and the Kruskal Wallis test when comparing more than 2 groups. For comparing categorical data, the Chi square ( $\chi^2$ ) test was performed. The Exact test was used instead when the expected frequency is less than 5. Odds ratio (OR) and 95% confidence interval (95%CI) were calculated for all studied 5A/6A polymorphism haplotypes and alleles between cases and controls. *p* values less than 0.05 were considered statistically significant. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

### 3. Results

#### 3.1. Characteristics of the study participants

This study included two groups; Group I: patients with acute myocardial infarction ( $n = 40$ ) including 9 (22.5%) females and 31 (77.5%) males with an age range of 35 to 67 years and a median of 53.5 years, [the patients' group was further sub-divided into 2 sub-groups according to age > 50 years  $n = 27$ (67.5%) and  $\leq 50$  years  $n = 13$  (32.5%)]. Group II: control group ( $n = 40$ ). Table 1 shows the laboratory data of the patients.

#### 3.2. MMP9 1562C > T and MMP3 5A/6A polymorphisms and AMI

The genotype and allele frequencies of the -1562C > T and the 5A/6A polymorphisms in the 40 AMI patients and 40 controls were compared (Table 2).

##### 3.2.1. MMP9

###### 3.2.1.1. Genotypes.

- CC genotype: a higher representation of the CC genotype was found among the control group as compared to the patients' group (Table 2).
- CT and TT genotypes: a statistically significant difference was present on comparing the patients' group and the control group as the CT or TT genotypes were only present in the patients ( $p = 0.005$ ), (Table 2).

###### 3.2.1.2. Alleles

- C allele: a higher representation was present on the control group (Table 2).

**Table 1** Laboratory data of patients.

Parameter	AMI <sup>a</sup> group ( <i>n</i> = 40)		
	Range	Normal range	Mean ± SD
<i>Cardiac enzymes</i>			
CK U/L	531–6562	0–206	1.8 ± 1.4
CK-MB U/L	421–1575	0–38	835.9 ± 314.5
LDH U/L	195–7838	40–150	1681.1 ± 1570.8
AST U/L	81–4670	5–35	545.5 ± 1.1
Troponin positive	In all patients		
ALT U/L	18–3490	5–35	310.8 ± 783.9
Creatinine mg/dl	0.5–3.2	0.2–1.2	1.2 ± 0.668

<sup>a</sup> AMI; acute myocardial infarction.

**Table 2** Genotyping of MMP-9 –1562C > T and MMP-3 5A/6A polymorphism and their allele frequency in the 2 studied groups.

Gene		AMI <sup>a</sup> patients	Control group	<i>P</i> value <sup>c</sup>
MMP-9 1562C > T		<i>n</i> <sup>b</sup> = 40	<i>n</i> = 40	
CC	<i>n</i> (%)	32 (80%)	40 (100%)	0.005
CT + TT	<i>n</i> (%)	8 (20%)	0 (0%)	
<i>Allele frequency</i>				
C		71 (88.75)	80 (100%)	0.006
T		9 (11.25)	0	
MMP-3 5A/6A polymorphism		<i>n</i> = 40	<i>n</i> = 40	
5A5A	<i>n</i> (%)	10 (25%)	1 (2.5%)	0.009
5A6A	<i>n</i> (%)	8 (20%)	13 (32.5%)	0.204
6A6A	<i>n</i> (%)	22 (55%)	26 (65%)	0.361
<i>Allele frequency</i>				
5A		28 (35%)	15 (18.75%)	0.02
6A		52 (55%)	65 (81.25%)	

<sup>a</sup> AMI; acute myocardial infarction.

<sup>b</sup> *n* = number.

<sup>c</sup> *p* value < 0.05 = significant.

- On comparing the T allele between patients and controls we had a statistically significant difference as the T allele was only present in the patients' group (*p* = 0.006) (Table 2).

control group (*p* = 0.02; OR = 2.333; CI = 1.130–4.820), (Tables 2 and 3).

- 6A allele: a higher representation of 6A allele was present in the control group.

### 3.2.2. MMP3

#### 3.2.2.1. Genotypes.

- 5A5A genotype: a statistically significant higher representation of the 5A5A genotype was found among the patients' group as compared to the control group (*p* = 0.009; OR = 13; CI = 1.576–107.233), (Tables 2 and 3).
- 5A6A genotype: the 5A6A genotype was underrepresented in the patients' group when compared to the control group (Tables 2 and 3).
- 6A6A genotype: a higher representation of the 6A6A genotype was present in the control group (Tables 2 and 3).

#### 3.2.2.2. Alleles

- 5A allele: a statistically significant higher level was present in the patients group when compared to the

Relation of risk factors to –1562C > T and the 5A/6A genotypes is shown in Table 4. There was no statistically significant association between the different variables and –1562C > T or the 5A/6A genotypes. Also there was no association between the values of different studied laboratory parameters (CK, CK-MB, LDH, AST, ALT, creatinine) and MMP3 or MMP9 polymorphisms' genotypes *p* value ≥ 0.05 Table 4.

## 4. Discussion

This study showed that the –1562C > T polymorphism in the promoter region of MMP-9 gene has a significant role in the development of an acute myocardial infarction. Comparison of genotypes and allele frequencies between patients and controls was highly significant (*p* value = 0.005 and 0.006, respectively) and showed an increased risk of development of MI among CT + TT genotypes as well as T allele carriers. Similarly Zhi et al. [16] found that CT/TT genotypes and T allele

**Table 3** Odds ratio (OR) of genotype and allele frequency of MMP3 polymorphism calculated on comparing patients with control group.

Genotype	Cases n(%)	Controls n(%)	OR <sup>a</sup>	95%CI <sup>b</sup> OR		P value <sup>c</sup>
				Upper	Lower	
5A5A	10 (25%)	1 (2.5%)	13.000	107.233	1.576	0.009
5A6A	8 (20%)	13 (32.5%)	0.519	1.438	0.187	0.204
6A6A	22 (55%)	26 (65%)	0.658	1.619	0.268	0.361
<i>Allele</i>						
5A	28 (35%)	15 (18.75 %)	2.333	4.820	1.130	0.02

<sup>a</sup> OR; odds ratio.<sup>b</sup> CI; confidence interval.<sup>c</sup> *p* value < 0.05 = highly significant.**Table 4** Association between genotypes in the studied polymorphisms and different clinical data.

Variable	MMP-9 1562C > T		P value	MMP-3 5A/6A polymorphism			P value
	CC	CT + TT		5A/5A	5A/6A	6A/6A	
<i>Age</i>							
> 50 years	21(65.6%)	6(75%)	1.0	5 (50%)	6(75%)	16(72.7%)	0.39
50 + < 50 years	11(34.4%)	2(25%)		5(50%)	2(25%)	6(27.3%)	
<i>Sex</i>							
Female	8(25%)	1(12.5%)	0.65	4(40%)	1(12.5%)	4(18.2%)	0.29
Male	24(15%)	7(87.5%)		6(60%)	7(87.5%)	18(81.5%)	
<i>Hypertension</i>							
Absent	7(21.9%)	0(0.0%)	0.31	2(20%)	2(25%)	3(13.6%)	0.74
Present	25(78.1%)	8(100%)		8(80%)	6(75%)	19(86.4%)	
<i>Diabetes</i>							
Absent	23(71.9%)	8(100%)	0.16	8(80%)	6(75%)	17(77.3)	0.97
Present	9(28.1)	0(0%)		2(20%)	2(25%)	5(22.7%)	

were associated with a significantly increased risk of MI in coronary artery disease patients [16]. Also Wang et al. [17] reported an association of the CT and the TT genotypes of MMP-9 polymorphism with the susceptibility to MI in the Uighur population of Xinjiang (China) [17].

Recently a meta-analysis by Wang et al. [18] including 18 potentially eligible articles tried to find an answer as to whether MMPs polymorphisms increase the risk of MI. This meta-analysis demonstrated that the MMP-9 -1562C > T polymorphism is a risk factor associated with increased MI susceptibility ( $p = 0.02$ ; OR = 1.14; 95%CI = 1.02–1.27), but this associations vary in different ethnic populations [18].

It was biologically acceptable that the variant allele of -1562 C > T polymorphism might increase the expression of MMP-9 by binding to the transcriptional repressor factor in the promoter [19] and the overexpression of MMP-9 was found in human atherosclerotic plaques and involved in rupture of the plaques [20]. This finding is similar to the results of a case-control study in a Korean population reporting that the -1562C > T polymorphism has a significant association with the development of MI [21]. Another autopsy study also reported that -1562T variant genotypes were related to the increased risk of MI significantly [22]. These results suggest that the MMP-9 -1562C > T polymorphism may play an important role in the development of MI due to plaque rupture.

Concerning the MMP3 5A/6A polymorphism; 5A/5A genotype was over represented in patients as compared to controls with a 13-fold increased risk of developing MI ( $p = 0.009$ ; OR = 13; CI = 1.576–107.233). A recent meta-analysis on the relation of MMP3 5A/6A polymorphism and MI, supported the association of the 5A allele with MI [18]. On the contrary, our data were different from a German case control study [23] which did not support the presence of an association between individual polymorphisms and haplotypes of MMP3 with MI. In addition, this study also performed a meta-analysis of the relationship of 5A/6A MMP3 polymorphism and coronary artery diseases using data obtained from their samples and those from available case-control studies, the results of the meta-analysis were also incompatible with a relationship of 5A/6A polymorphism in the MMP3 promoter region and atherosclerotic diseases of the coronary arteries. In subgroup analyses, evidence was obtained to suggest different risk effects of 5A/6A polymorphism between populations of European descent and East Asians (Japanese and Chinese) [23] The combination of samples from Europe and East Asia contributed significantly to the high degree of heterogeneity across studies. This ethnicity-related heterogeneity may reflect the striking differences that exist in the allele frequencies of 5A/6A MMP3 polymorphism between populations of whites and other ethnic groups. There is evidence for the action of positive selection, beginning approximately 24,000 years ago, increasing the frequency of the 5A allele to an average of



50% in Europe but not elsewhere, including East Asia where the frequency of the 5A allele is on average 15% [24].

In conclusion, this study supported the association of a functional polymorphism in the promoter region of the -1562C > T of the MMP9 gene and AMI in the Egyptian population. Further study on a larger population will be required to confirm this polymorphism as a potential novel genetic marker for a plaque rupture in Egyptians. Similarly, our study supported the presence of the 5A/5A genotype of MMP3 gene promoter as a risk factor of AMI in Egyptian patients. Meanwhile, the race selection should be paid more attention since the pathogenesis of a disease might have different bases in different racial population groups.

### Acknowledgement

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