Matrix Metalloproteinase 3 Gene Polymorphism and Its Level Predict Morbidity After Acute Myocardial Infarction

Tarek A. Abd El-Aziz, MD, AFACC,1 and Randa H. Mohamed, MD2

From the 1Cardiology Department, and 2Medical Biochemistry Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt.

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ABSTRACT

Objectives: Matrix metalloproteinase is responsible for ventricular remodeling after acute myocardial infarction (MI). The purpose of the present study was to determine whether the matrix metalloproteinase 3 (MMP-3) polymorphism and its level predict morbidity after acute MI (AMI).

Methods: We studied 112 patients with AMI and 140 controls. All patients were followed for AMI complications during their hospitalization and 6 months after. Serum MMP-3 was measured. MMP-3–1612 5A/6A polymorphism was genotyped by polymerase chain reaction.

Results: We observed that the serum MMP-3 levels were significantly increased in patients with AMI with morbidity compared with patients without complications. Also, MMP-3 levels in patients with AMI carrying 5A/5A were elevated compared with those carrying 6A/6A. The frequencies of 5A/5A genotypes were significantly increased in patients with AMI compared with controls, and patients with AMI carrying 5A/5A had a fivefold increased risk of developing morbidity. The impairment of left ventricular function (ΔFS [fractional shortening] and ΔEF [ejection fraction]) was observed more in the 5A/5A genotype compared with the 6A/6A genotype. A significant inverse correlation between predischarge MMP-3 levels and FS and EF was found at 6 months follow-up.

Conclusions: MMP-3 polymorphism has a significant association with the risk of developing morbidity after AMI. Higher predischarge MMP-3 levels are associated with left ventricular dysfunction after AMI.

Acute myocardial infarction (AMI) is currently one of the most important health problems in many countries. AMI has devastating consequences in the early phase, such as cardiac rupture, and in the chronic phase, such as chronic heart failure. Dynamic expression and activation of matrix metalloproteinases (MMPs) associated with morphologic changes occur after myocardial infarction (MI) at both infarcted and peri-infarcted regions. These changes are known as the cardiac remodeling process. Cardiac remodeling involves changes in cardiac myocytes and in the extracellular matrix (ECM); changes in the ECM result in a loss of normal structure and function of the myocardium. 1

MMPs are a large family of zinc-dependent enzymes that are capable of degrading most components of the ECM and contribute to both normal and pathologic tissue remodeling. 2 In post-AMI, there are multiple reports of MMP levels correlating with echocardiographic parameters of left ventricular (LV) function, indicating they possibly contribute toward remodeling. 3-5

MMP-3 (stromelysin 1), a member of the MMP family, has an important role in the turnover of ECM and is known to degrade collagen types III, IV, IX, and X; proteoglycans; laminin; elastin; and fibronectin. It is also involved in the activation of other MMPs (eg, pro–MMP-1, -8, -9, and -13), as well as in autoactivation of pro–MMP-3. 6

The gene coding for MMP-3 is located on the long arm of chromosome 11 in regions 11q22.2 to 22.3. A single-nucleotide polymorphism has been identified in the promoter region of the MMP-3 gene, located 1,612 base pairs (bp) upstream of the transcription start site, resulting in one allele having a run of five adenine nucleotides (5A) and the other
having six adenine nucleotides (6A). The 5A allelic stromelysin 1 promoter has been shown to have greater transcription activity than the 6A allelic promoter.\(^7\) Apparently, this difference is due to preferential binding of a transcription repressor to the 6A allele.\(^8\)

The purpose of the present study was to determine whether the MMP-3 polymorphism and its level predict morbidity after AMI.

### Materials and Methods

The study population included 112 male patients with AMI, with a mean ± SD age of 57.2 ± 10.9 years. They were classified into two subgroups: AMI without complications group (n = 70) and AMI with morbidity group (n = 42), who had complications in the form of heart failure, arrhythmia (atrial fibrillation, ventricular tachycardia, and heart block), LV thrombus, myocardial aneurysm, cardiogenic shock, cardiac arrest, and reinfarction.

AMI was diagnosed in the presence of two of the following criteria: persistent angina pectoris for 20 or more minutes and ST segment elevation of 2 mm or more in two or more contiguous precordial leads or the presence of a left bundle branch block. AMI was later confirmed based on the elevation of cardiac enzymes of more than twice the upper limit of the normal range.\(^9\)

The exclusion criteria included unstable angina, rheumatic or congenital heart disease or cardiomyopathy, active malignancy, inflammation, or connective tissue disease.

Follow-up of all patients for 6 months for complications and echocardiographic parameters of LV function was performed.

The control group comprised 140 healthy volunteer male subjects with no family history of coronary artery disease (CAD) or stroke who were matched for ethnicity and age and from the same geographical region. The ethical committee of Zagazig University approved this study, and written informed consent was obtained from all participants before their inclusion in this work.

### Laboratory Methods

#### Serum Assays

Samples were obtained at the time the patient was admitted to the intensive care unit. These samples were specifically obtained for this study. Blood was drawn under standardized conditions and stored at -80°C. Serum cardiac markers (CK, CK-MB isoenzyme, and cardiac troponin) and serum MMP-3 levels were measured using a commercially available enzyme-linked immunosorbent assay (RayBio Human MMP-3 ELISA Kit Parkway, Norcross, GA). Serum lipid levels were measured.

DNA extraction: Genomic DNA was extracted from EDTA whole blood by a standard method according to the manufacturer’s instructions (QIAamp Blood Kit; Qiagen GmbH, Hilden, Germany).

Genotyping of MMP-3–1612 5A/6A polymorphism (rs3025058): Genotyping of MMP-3 was carried out by a polymerase chain reaction (PCR)–based restriction digestion method.\(^6\) The specific primer for detecting -1612 5A/6A polymorphism was 5’-GGTTTCATTCTTCTTGTAGGGGGGAAAGA-3’ and 5’-CTTCTTGGAATTCACACTACTGCCACC-3’. The PCR was performed in a 50-μL reaction mixture containing 10 μL genomic DNA, 30 μL one-step PCR mixture (1 unit Taq polymerase, 10 mmol/L KCl, 10 mmol/L [NH₄]₂SO₄, 20 mmol/L Tris-HCl pH 8.75, 0.1% Triton X-100, 0.1 mg/mL bovine serum albumin, and 200 μM deoxyribonucleoside triphosphates), and 2 μL of each primer (BioBasic, Ontario, Canada) and 6 μL double-distilled H₂O. Amplification conditions included initial denaturation at 95°C for 5 minutes followed by 30 cycles of 94°C for 60 seconds, 60°C for 60 seconds and 72°C for 60 seconds, and a final extension of 72°C for 10 minutes. After digestion with Psy I, the 6A/6A genotype generated a larger fragment of 130 bp, while 5A/5A generated a shorter fragment of 110 bp. Heterozygote 5A/6A demonstrated a band at 130 bp and 110 bp.

### Echocardiogram Assessment

All patients underwent echocardiographic examination before discharge and at 6 months after AMI. All measurements were performed in accordance with the recommendations of the American Society of Echocardiography/European Association of Echocardiography.\(^10\)

Echocardiographic assessment was carried out using a Sonos 5500 with a 2- to 3-MHz transducer (Alpha Park, Cleveland, OH). LV end systolic volume, LV end diastolic volume, and LV ejection fraction (LVEF) were estimated using the biplanar modified Simpson’s rule from apical two- and four-chamber views. LV wall motion index score was measured using a standard 16-segment model from parasternal long- and short-axis and apical two- and four-chamber views. Each LV segment is scored as follows: 1 = normal, 2 = hypokineic, 3 = akinetic, 4 = dyskinetic, and 5 = aneurysmal. The total divided by the number of segments analyzed gives an overall score, with higher values indicating more impaired LV function.

### Statistical Analysis

The descriptive data were expressed as the mean ± SD, and categorical variables were presented using frequency counts. The means of groups were compared by one-way analysis of variance. The χ² test was used to compare the
categorical variables between the groups. The MMP-3 gene variants under investigation were evaluated for deviation from Hardy-Weinberg equilibrium analysis by comparing observed and expected genotype frequencies by the \( \chi^2 \) test in case and control groups. In addition, we calculated the odds ratios (ORs) and 95% confidence intervals (CIs) regarding the presence of AMI or morbidity with respect to the existence of polymorphism. Correlations between predischarge levels of MMP-3 and echocardiographic predischarge parameters and MMP-3 level at follow-up were performed using the Pearson correlation test. A difference was considered significant at \( P < .05 \). All data were evaluated using SPSS version 10.0 of Windows (SPSS, Chicago, IL).

Results

A total of 112 consecutive patients were analyzed at admission and at the 6-month follow-up. The clinical and laboratory data of the study population are presented in Table 1.

Serum MMP-3 Levels in AMI Without Complications Group and AMI With Morbidity Group

Serum MMP-3 levels were significantly increased in the patients with AMI with morbidity (18.7 ± 2.9 ng/mL) compared with patients without complications (16.5 ± 1.8 ng/mL) (\( P < .001 \)).

Serum MMP-3 Levels (ng/mL) in Study Population According to MMP-3 Genotype

Serum MMP-3 levels were significantly increased in 5A/5A (18.9 ± 2.9 ng/mL) compared with 5A/6A individuals (16.9 ± 2.6 ng/mL) and 6A/6A homozygotes (16.8 ± 2.3 ng/mL) (\( P < .001 \)), with no significant difference between 5A/6A and 6A/6A genotypes.

Distribution of MMP-3 Genotype in All Studied Groups

The distribution of the MMP-3 polymorphism in the control and AMI groups was in accordance with the Hardy-Weinberg equilibrium (\( \chi^2 = 0.17 \) and 0.13; \( P > .05 \)), suggesting that our sample was representative of the population (Table 2). The frequencies of 5A/5A genotypes were significantly increased in patients with AMI compared with controls. Those with the 5A/5A genotype had a twofold increased risk of developing AMI (OR, 2.2; 95% CI, 1.1-4.5; \( P = .026 \)). The frequency of the 5A allele in the group with AMI was significantly higher than that in the controls (OR, 1.4; 95% CI, 1.2; \( P = .036 \)).

The genotype distribution of MMP-3 polymorphisms among AMI patients without complications and AMI patients with morbidity was significantly different. The frequency of genotype 5A/5A in the group with morbidity was 40.5%, which was significantly higher than that in the group without complications (15.7%) (OR, 5.1; 95% CI, 1.6-15.8; \( P = .004 \)). The frequency of the 5A allele in the group with morbidity...
was 61.9%, which was significantly higher than that in the group without complications (41.4%) (OR, 2.3; 95% CI, 1.3-3.9; P < .002).

Echocardiographic Parameter Difference Between Predischarge and Follow-up According to MMP-3 Genotype

Fractional shortening (FS) and ejection fraction (EF) were decreased at the 6-month follow-up in the three genotypes. FS and EF were significantly decreased in 5A/5A compared with 6A/6A. The Wall Motion Score Index (WMSI) was increased at the 6-month follow-up in 5A/5A and 5A/6A. Early diastolic LV filling velocity/late diastolic LV filling velocity (E/A) was increased at the 6-month follow-up in the three genotypes. ΔWMSI and ΔE/A were significantly increased in 5A/5A compared with 6A/6A.

Correlations Between Predischarge Levels of MMP-3 and Echocardiographic Predischarge Parameters and at Follow-up

Predischarge levels of MMP-3 correlated directly with WMSI, EF, and correlated inversely with the 6-month follow-up FS (r = -0.25, P = .03) and EF (r = -0.29, P = .01). No correlation was found between MMP-3 and echocardiographic measures at predischarge.

Discussion

The present study found that patients with AMI carrying the 5A/5A genotype have a fivefold increased risk of developing morbidity compared with patients with AMI carrying the 6A/6A genotype. Also, we found that serum MMP-3 levels were significantly increased in patients with AMI with morbidity compared with patients with AMI without complications. Thus, the present study suggests that MMP-3 genotyping and serum levels could provide important clinical implications. In the future, MMP-3 genotyping and serum levels may be used as a possible test for prediction of morbidity after AMI.

Several past studies have provided evidence to suggest that increased myocardial MMP activity exists with severe forms of congestive heart failure (CHF). The increased MMP-3 level and activity observed with the development of CHF may play a contributory role in LV myocardial collagen remodeling directly through the degradation of collagen matrix components as well as the activation of endogenous myocardial MMPs.

FS and EF in the three genotypes of MMP-3 decreased at the 6-month follow-up compared with FS and EF at admission. The impairment of LV function (ΔFS and ΔEF) was observed more in the 5A/5A genotype compared with the 6A/6A genotype. The increase of E/A in the 5A/5A genotype at the 6-month follow-up compared with E/A at admission is probably due to pseudo-normalization of LV diastolic dysfunction. Thus, the 5A/5A genotype is associated with LV systolic and diastolic dysfunction at the 6-month follow-up. MMP-3 has previously been associated with dilatation of the left ventricle. Coker et al found a correlative relationship between LV geometry and myocardial MMP activity. However, there was no significant correlation between MMP-3 concentration in blood and LV parameters, apart from LVEF, in the study by Berezin and Samura.

In the present study, we observed that MMP-3 levels were more associated with the 5A/5A genotype. Our results are in agreement with the reports of Mizon-Gerard et al, Medley et al, and Ghaderian et al. Also, Ye et al found that MMP-3 expression in vascular tissues is higher in individuals carrying the 5A allele than in individuals of the 6A/6A genotype. However, Gao and Li reported no significant difference in the peripheral levels of MMP-3 when compared between genotypes in controls as well as the CAD group. Samnegard et al and White et al have
shown increased serum concentration of MMP-3 with the 6A allele. The difference in the MMP-3 levels in the study of Shalia et al\textsuperscript{6} did not reach statistical significance when 5A/5A + 5A/6A was compared with 6A/6A genotypes.

In the present study, we observed that the frequencies of 5A/5A genotypes were significantly increased in patients with AMI compared with controls. The association of the 5A allele with MI was reported in Egyptian patients,\textsuperscript{22} Han-Chinese patients from China,\textsuperscript{23} young (age 45 years) Han-Chinese patients from Taiwan,\textsuperscript{24} Japanese populations,\textsuperscript{25} and whites.\textsuperscript{26} However, a genome-wide association study looking at 112 polymorphisms in 71 candidate genes in 4,152 Japanese people found an association between the 6A allele and MI, although this was confined to women.\textsuperscript{27} In contrast with all the above findings, others investigated the MMP-3 gene 5A/6A polymorphism in relation to the risk of MI but did not detect any effect of the MMP-3 5A allele on the risk of MI.\textsuperscript{6,19,28} Also, Dalepiane et al\textsuperscript{29} found no significant differences in genotype frequencies between patients with CAD and controls.

The most common cause of MI is coronary atherosclerotic plaque rupture or erosion, resulting in exposure to thrombus formation.\textsuperscript{30} The association between the increase in MMP-3 level and MI has been investigated by different authors. In the present study, we observed that MMP-3 levels were elevated in patients with AMI compared with controls. Our results are in agreement with the reports of Gao and Li\textsuperscript{15} and Shalia et al\textsuperscript{6} for patients with AMI. In contrast, Samnegard et al\textsuperscript{20} showed that postinfarction patients have lower circulating levels of MMP-3 than do healthy individuals.

In the present study, we found a significant inverse correlation between predischarge MMP-3 level and FS and EF at the 6-month follow-up. Kelly et al\textsuperscript{3} showed that MMP-3 concentration prior to discharge had a direct association with LV function and volume measures in the follow-up period. They observed a consistent association for higher circulating MMP-3 in the days following AMI with adverse LV remodeling and dysfunction. They suggested a pathophysiologic role for MMP-3 in the process of LV remodeling.

Since MMP-3 is considered to play an important role in the degradation of matrix proteins in atherosclerotic lesions and its level is higher in individuals carrying the 5A allele than in individuals with the 6A/6A genotype, a possible explanation for our finding is that those with the 5A/5A genotype may have an increased risk of plaque rupture and subsequent MI. We conclude that the MMP-3 polymorphism has a significant association with the risk of developing morbidity after AMI. Higher predischarge MMP-3 levels are associated with LV dysfunction after AMI.

The study did not include women because there were sex differences; also, women have significantly lower serum levels of MMP-3 than do men. However, another study could include both sexes and then analyze them separately. We did not perform a correlation between MMP-3 level and its polymorphism with MI size or MI site. However, this can be performed in another study.

**References**


**Corresponding author:** Randa H. Mohamed, Faculty of Medicine, Zagazig University; randahussiny@yahoo.com.


