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rs17576 polymorphism of matrix metalloproteinase-9 in predicting coronary artery disease severity and its adverse outcome

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Abstract

BACKGROUND: The role of matrix metalloproteinases in developing ischemic heart disease has been suggested. We investigated the effect of the *MMP-9* gene polymorphism rs17576 on the severity of coronary artery disease and outcomes in affected individuals.

METHODS: A total of 654 patients suspected of having coronary artery disease were assessed. Real-time PCR was performed for *MMP-9* (rs17576) genotyping, and ELISA was used to assess *MMP-9* plasma levels. The patients were followed up for one year.

RESULTS: Coronary angiography showed coronary artery involvement in 28% of patients. The frequencies of AA, AG, and GG genotypes of rs17576 in the group without coronary artery involvement were 5.5%, 31.4%, and 63.1%, respectively, while in those with coronary artery disease, the frequencies were 55.2%, 29.5%, and 15.3%, respectively, showing a significant difference ($p < 0.001$). The frequency of the major allele (G allele) in the normal group and the groups with single-, two-, and three-coronary involvement was 21.1%, 65.4%, 61.8%, and 85.0%, respectively, indicating a significant difference ($p < 0.001$). The mean serum level of *MMP-9* was 2,963 ± 1,077 pg/ml in the group with coronary artery disease and 2,145 ± 926 pg/ml in the group without coronary involvement, with a significant difference between the two groups ($p = 0.008$). The presence of the A allele of rs17576 was associated with an increased hazard of one-year mortality in the coronary artery disease group (HR = 5.764, $p < 0.001$).

CONCLUSION: Tracking the rs17576 polymorphism of the *MMP-9* gene can predict coronary artery disease severity and its long-term poorer outcome.

Keywords: Matrix Metalloproteinase-9; Single Nucleotide Polymorphism; Coronary Artery Disease; Outcome

Introduction

Atherosclerosis is a complex multifactorial pathophysiology of the coronary arteries, ultimately leading to coronary artery stenosis and acute coronary syndrome[1,2]. This process includes a cascade of various inflammatory, oxidative, and cellular processes, including smooth muscle cell proliferation, lipid accumulation, platelet aggregation, cell apoptosis, endothelial fibrosis, and necrosis[3]. The activation of these processes is under the control and induction of a set of enzymes and proteins encoded by specific genes.

Matrix metalloproteinases (MMPs) are specific zinc-dependent enzymes with proteolytic activities, whose alteration is closely linked to various vascular pathologies such as endothelial dysfunction, smooth muscle cell migration, vascular calcification, and endothelial cell apoptosis[4,5]. This set of abnormalities plays an essential role in the process of atherosclerotic plaque formation, followed by plaque rupture and the occurrence of acute ischemic heart attacks[6-8]. Accordingly, the close relationship between changes in MMP expression and dysfunction of MMP molecules with coronary heart disease has been comprehensively investigated[9,10].

MMP-9, also known as gelatinase B, is secreted by macrophages and plays a major role in the breakdown of the extracellular matrix [11]. In fact, its hyperactivation can result in proteolysis of type IV collagen, which is a main component of the basement membrane of coronary arteries[12-14]. In addition, *MMP-9* appears to play a role in remodeling processes related to atherosclerosis and plaque rupture[15]. According to the literature, the level of this marker is considerably increased following tissue injuries, especially vascular injuries, as well as after the activation of inflammatory processes[16,17].

After vascular injury, there is an increase in *MMP-9* expression, particularly in inflammatory atherosclerotic lesions. However, the results of different studies conducted in various communities regarding the role of this marker in the process of coronary atherosclerosis—and consequently, its role in the occurrence of coronary heart disease—have been contradictory.

The rs17576 polymorphism is a single nucleotide polymorphism (SNP) located in the promoter region of the *MMP-9* gene. This SNP has been studied in relation to various conditions, including cancer, cardiovascular diseases, and inflammatory disorders[18,19]. Different alleles of rs17576 may influence the expression or activity of *MMP-9*, potentially affecting disease susceptibility or progression in individuals carrying those alleles. However, the specific effects of this SNP can vary depending on the context of the disease or condition being studied, as well as other genetic and environmental factors. Therefore, further research is often needed to fully understand the implications of genetic variations like rs17576 in different outcomes[20].

We aimed to examine the association of the rs17576 polymorphism with the development and severity of coronary artery disease in a sample of the Turkish population. We then examined the association between rs17576 genotypes and serum levels of *MMP-9* protein. Additionally, we assessed whether the evaluation of this protein's levels could predict the severity of coronary artery involvement.

Materials and Methods

Study group

In this cohort study, 654 patients suspected of having coronary artery disease who were referred to a referral heart center in Istanbul, Turkey, between 2023 and 2024 and underwent coronary angiography were included. Suspicion of coronary artery disease was defined as having either typical angina symptoms or atypical chest pain with exercise-induced myocardial ischemia detected by an exercise test[20]. A total of 183 of the 654 patients were diagnosed with coronary artery disease (CAD) according to the angiography reports and were named as the CAD group. The remaining 471 patients, whose coronary angiograms were normal, were designated as the group without CAD.

To ensure the accuracy of the recorded

angiography results, the relevant report, along with the angiography CD, was reviewed and judged by a second cardiologist (blinded to the first cardiologist). If there was agreement between the two observers, the case was included in the study. According to the study by Pogorielova et al.[21], considering a relative precision of 50%, a confidence level of 95%, a study power of 90%, an expected prevalence of the major allele of the polymorphism in the control group of 5.0%, and an odds ratio of 0.54 related to the analysis between *MMP-9* rs17576-SNP and CAD development mentioned in the referenced study, the minimum number of samples required for the study was estimated to be at least 466. Thus, the number of samples included in the study provided adequate statistical power.

The three main indications for coronary angiography were the presence of symptoms suspected to indicate coronary involvement (such as typical chest pain), elevated cardiac enzyme levels, and abnormal electrocardiogram changes suggestive of cardiac ischemia. Patients with previous cardiac events or those who had undergone cardiac revascularization were excluded from the study. The presence of systemic disorders such as chronic obstructive pulmonary disease, connective tissue disorders, and any malignancies was also considered exclusion criteria. Considering the potential impact of COVID-19 on the risk of ischemic heart disease, patients who had a history of COVID-19 or had been hospitalized due to this disease were also excluded from the study.

All procedures were in line with the Declaration of Helsinki (1964), and all participants provided written informed consent before participation in the study.

Determination of coronary artery involvement

The baseline information, including patients' characteristics, cardiovascular risk profiles, clinical manifestations, oral medications, and history of any surgical interventions, was collected by interviewing the patients or their caregivers. The patients were scheduled for coronary angiography to assess the presence of coronary involvement and its severity according to the number of involved coronary arteries, as well as determining the Gensini score. To determine the Gensini score, the number, location, and degree of stenosis in each coronary artery were considered. The calculation of this score was previously described in detail [22]. In this context, coronary artery disease was defined as more than 50% stenosis in the luminal diameter of a coronary artery or its first-order branch.

Determination of MMP-9 genotypes and MMP-9 plasma levels

Blood samples were collected from each subject immediately before coronary angiography for the determination of rs17576 genotyping and *MMP-9* plasma levels. In this regard, the collected blood sample was first maintained at room temperature for 30 minutes and then centrifuged at 3,000 rpm at 4°C for 15 minutes. Serum was then poured into a polypropylene tube and frozen at -80°C until the time of analysis.

Real-time PCR followed by restriction fragment length polymorphism (RFLP) was performed for *MMP-9* (rs17576) genotyping, and ELISA (Calbiochem® *MMP-9* ELISA Kit) was used to assess the plasma level of *MMP-9*. First, the GeneJET Genomic DNA Purification Kit was used to extract DNA from whole venous blood samples using a DNA extraction kit (Promega, Beijing, China). Real-time PCR was performed for *MMP-9* (rs17576) genotyping using the forward primer 5'-TCACCCTCCCGCACTCTGG-3' (position on the gene: 3191-3208, length: 18 bp) and the reverse primer 5'-CGGTCGTAGTTGGCGGCGGTGG-3' (position on the gene: 4958-4975, length: 18 bp). The primers were designed using Beacon Designer version 8.0 (Premier Biosoft, Palo Alto, USA). The Primer-BLAST tool was used to confirm the specificity of the designed primers, and the sequences were confirmed by sequencing before application in PCR setup (Figure 1). The results of the sequencing examination were analyzed to confirm the presence of the *MMP-9* rs17576 gene polymorphism.

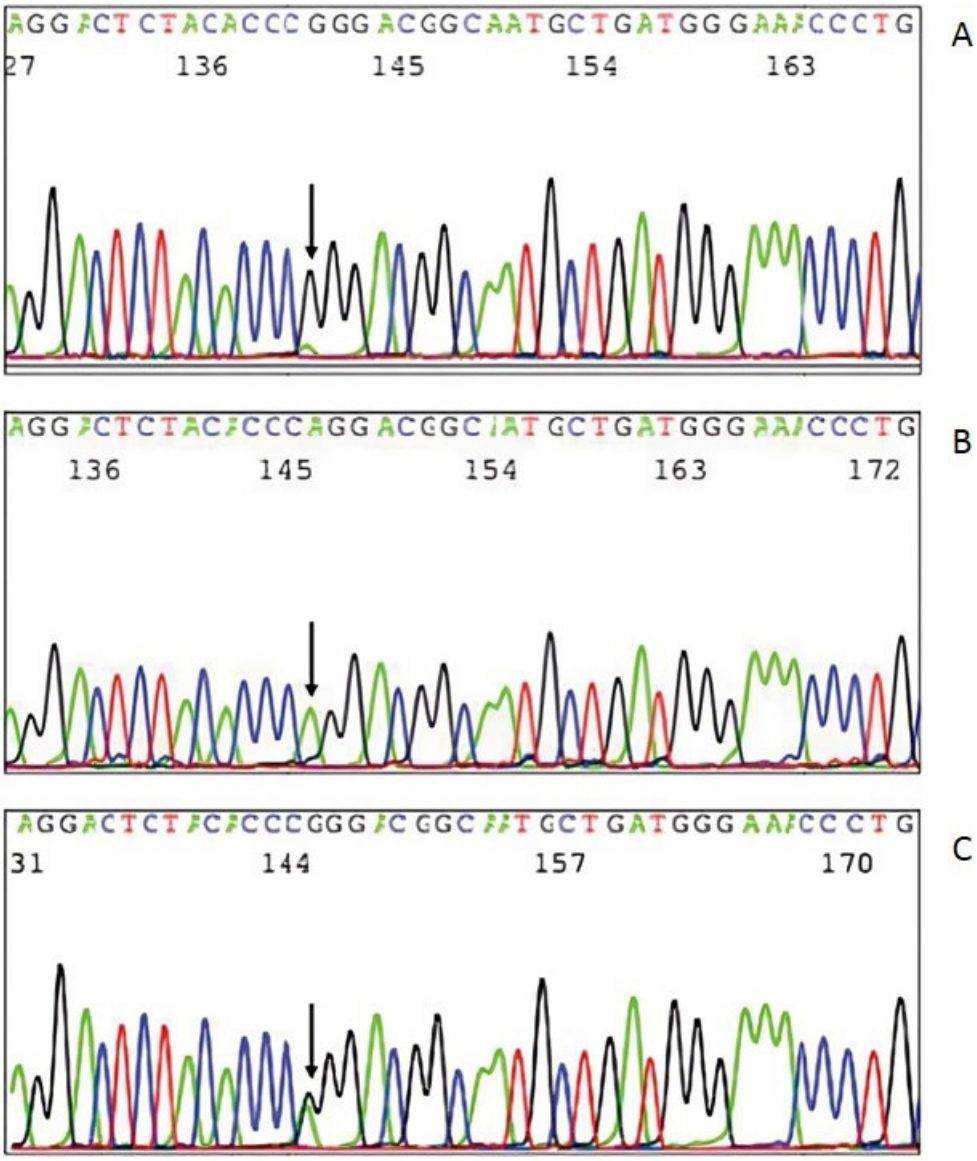

Figure 1. The results of sequencing for confirming the presence of the MMP-9 rs17576 gene polymorphism (A indicates the situation of the minor allele G at the polymorphic site instead of A; B shows the major allele A; C indicates the presence of the heterozygous A/G genotype)

The *MMP-9* rs17576 polymorphism was amplified from 100 ng of genomic DNA in a 15-µL PCR reaction. The PCR assay was set as follows: denaturation at 95°C for 45 seconds, treatment at 95°C for 15 seconds, and at 60°C for 30 seconds, with a total of 45 cycles. RT-PCR was performed using the Light Cycler 480 II instrument (Roche, Germany). For quality control of RT-PCR, 5% of all samples were duplicated within and across plates. The data were then processed using the 7500 Fast Real-time PCR Software.

In the RFLP phase, the MspI restriction enzyme was used for genotyping the rs17576 polymorphism, producing the following fragments: AA: 300 bp, AG: 300 + 170 + 130 bp, GG: 170 + 130 bp. ELISA (Calbiochem® *MMP-9* ELISA Kit) was performed to measure *MMP-9* plasma concentration.

According to recommendations released by the International Council for Harmonization (ICH) (24) for validation and precision of the ELISA, the samples were first prepared at least in

Table 1. Baseline characteristics of study patients with and without CAD

Characteristics	Group with CAD (n = 183)	Group without CAD (n = 471)	P-value
Male gender, %	107 (58.5) *	246 (52.2)	0.126
Mean age, year	62.36±11.12	56.12±9.26	0.001
Mean body mass index, kg/m²	27.46±4.23	24.12±3.89	0.002
Cardiovascular risk profiles, %			
Hypertension	106 (57.9)	182 (38.6)	0.001
Diabetes mellitus	84 (45.9)	86 (18.2)	0.008
Hyperlipidemia	95 (51.9)	77 (16.3)	0.001
Smoking	26 (14.2)	38 (8.1)	0.026
Family history of cardiac disease	86 (47.0)	42 (9.2)	0.001
Cerebrovascular events	12 (6.5)	22 (4.7)	0.297
Renal dysfunction	10 (5.4)	26 (5.5)	0.789
Oral medications			
Aspirin	46 (25.1)	87 (18.5)	0.097
Beta-blockers	82 (44.9)	145 (30.8)	0.036
Calcium blockers	16 (8.7)	42 (8.9)	0.234
Statins	90 (49.2)	98 (20.8)	0.032
Anti-glycemic drugs	77 (42.1)	122 (25.9)	0.012
Diuretics	42 (22.9)	126 (26.7)	0.456
ACE-inhibitors	46 (25.1)	77 (16.3)	0.224
Serum hemoglobin level, g/dL	11.46±1.26	11.53±1.33	0.856
Serum fasting blood sugar, mg/dl	98.12±10.26	87.52±9.29	0.008
Serum triglyceride level, mg/dl	155.24±12.13	102.01±10.56	0.002
Serum HDL level, mg/dl	32.26±9.89	46.52±12.10	0.001
Serum LDL level, mg/dl	122.12±12.14	98.99±10.78	0.098
Serum creatinine level, mg/dl	1.02±0.26	0.98±0.12	0.452

* The results were indicated as number (percentage) or mean±standard deviation

four different percentages, including the lower limit of quantitation (LLOQ), 30–50% of the calibration curve range, and at least 75% of the upper limit of quantitation (ULOQ). After running each of these, the coefficient of variation (%CV) was calculated using the following formula: %CV = (standard deviation / sample amount) × 100. To ensure an accurate ELISA test, ICH specifies that %CV should not exceed 15%.

Following-up the patients

To assess the one-year follow-up data, the patients were followed up by phone regarding adverse cardiovascular events, including cardiac-related death and MACE (defined as the presence of at least one of the following events: mortality or the need for cardiac interventions such as coronary artery bypass grafting (CABG) or percutaneous coronary intervention (PCI)). The study endpoint was to first determine the association of rs17576 detection with coronary artery disease and its severity and then to examine the value of this SNP in predicting one-year patient outcomes.

Statistical analysis

Data were analyzed using IBM SPSS Statistics software version 21.0 (Armonk, NY: IBM Corp.). The Kolmogorov-Smirnov test was used to determine the normality of the continuous variables, revealing that all continuous variables had a normal distribution. Thus, continuous variables were presented as mean ± standard deviation. The independent t-test was used to compare continuous variables between the groups. Categorical variables were presented as frequencies and percentages and compared between the groups using the chi-square test.

The multivariate logistic regression model was employed to determine the association between rs17576 polymorphism and the presence of coronary artery disease. The Cox proportional hazard model was also used to analyze the association between the presence

of gene polymorphism and one-year events, with the hazard ratios then displayed. In this model, we included covariates such as gender, age, classic risk factors for coronary artery disease, the presence of multi-vessel disease, and baseline cardiac functional status based on left ventricular ejection fraction (LVEF). P-values of <0.05 were considered statistically significant.

Results

Study Characteristics

Table 1 presents the baseline characteristics of the study population. A comparison of the two groups revealed that individuals with coronary artery disease had a significantly higher mean age, body mass index (BMI), and a higher prevalence of hypertension, diabetes mellitus, hyperlipidemia, smoking, and a family history of ischemic heart disease.

With regard to laboratory parameters, those with coronary artery disease exhibited significantly elevated serum levels of fasting glucose and triglycerides, along with a reduction in HDL levels. According to coronary angiography findings, coronary artery involvement was demonstrated in 183 (28.0%) patients, with single-vessel disease in 25 (13.7%) patients, two-vessel disease in 98 (53.6%) patients, and three-vessel disease in 60 (32.7%) patients.

Association of the MMP-9 rs17576 A>G Polymorphism with CAD and severity of CAD

With regard to the findings of rs17576 polymorphism genotyping, the frequency of AA, AG, and GG genotypes in the group without coronary artery disease was determined as 5.5% (n = 26), 31.4% (n = 148), and 63.1% (n = 297), respectively, while the frequency of these genotypes in the group with coronary artery disease was determined as 55.2% (n = 101), 29.5% (n = 54), and 15.3% (n = 28), respectively (Figure 2).

As indicated in Table 2, the frequency of the AA genotype was closely associated with the number of diseased coronary arteries. Also, the frequency of the A allele was significantly associated with a higher number of involved coronary arteries. The association between the presence of the A allele and multi-vessel coronary involvement was also assessed, adjusting for gender, indicating a significant difference in A allele frequency between the two groups with single-vessel and multi-vessel involvement (odds ratio: 1.226, 95% confidence interval: 1.112–3.126, p = 0.002).

Overall, the mean Gensini score in the subgroups of coronary artery disease patients with AA, AG, and GG genotypes was 77.24 ± 8.89, 28.56 ± 6.96, and 12.66 ± 4.22, respectively. The mean serum level of *MMP-9* was 2,963 ± 1,077 pg/ml in the group with coronary disease and 2,145 ± 926 pg/ml in the group without coronary involvement, showing a significant difference between the two groups (Figure 3).

Regarding the serum levels of *MMP-9* according to the rs17576 genotypes, the value of this biomarker in the subgroups with AA, AG, and GG genotypes was 2,926 ± 1,033 pg/ml, 2,116 ± 989 pg/ml, and 1,826 ± 889 pg/ml, respectively, indicating a significant difference (p = 0.001) (Figure 4).

The association of rs17576 genotyping and patients' outcome

Within one year of follow-up, a total of 22 deaths and 38 MACE events were recorded. Comparing the frequency of AA, AG, and GG genotypes in the non-survived and survived subgroups (Table 3) showed a higher frequency of the AA genotype in the non-survived group. Additionally, the frequency of the A allele was higher in the non-survived group than in the survived group. No association was found between rs17576 genotyping and the occurrence of one-year MACE (Table 3).

The mean serum level of *MMP-9* in non-survived and survived patients was 2,866 ± 1,026 pg/ml and 1,998 ± 912 pg/ml, respectively, indicating a significant difference between the two groups. According to the Cox proportional hazard analysis, accounting for baseline confounders (Table 4), the presence of the A allele of rs17576 was associated with a higher hazard of one-year mortality (HR = 5.764, 95% CI: 1.659–20.021).

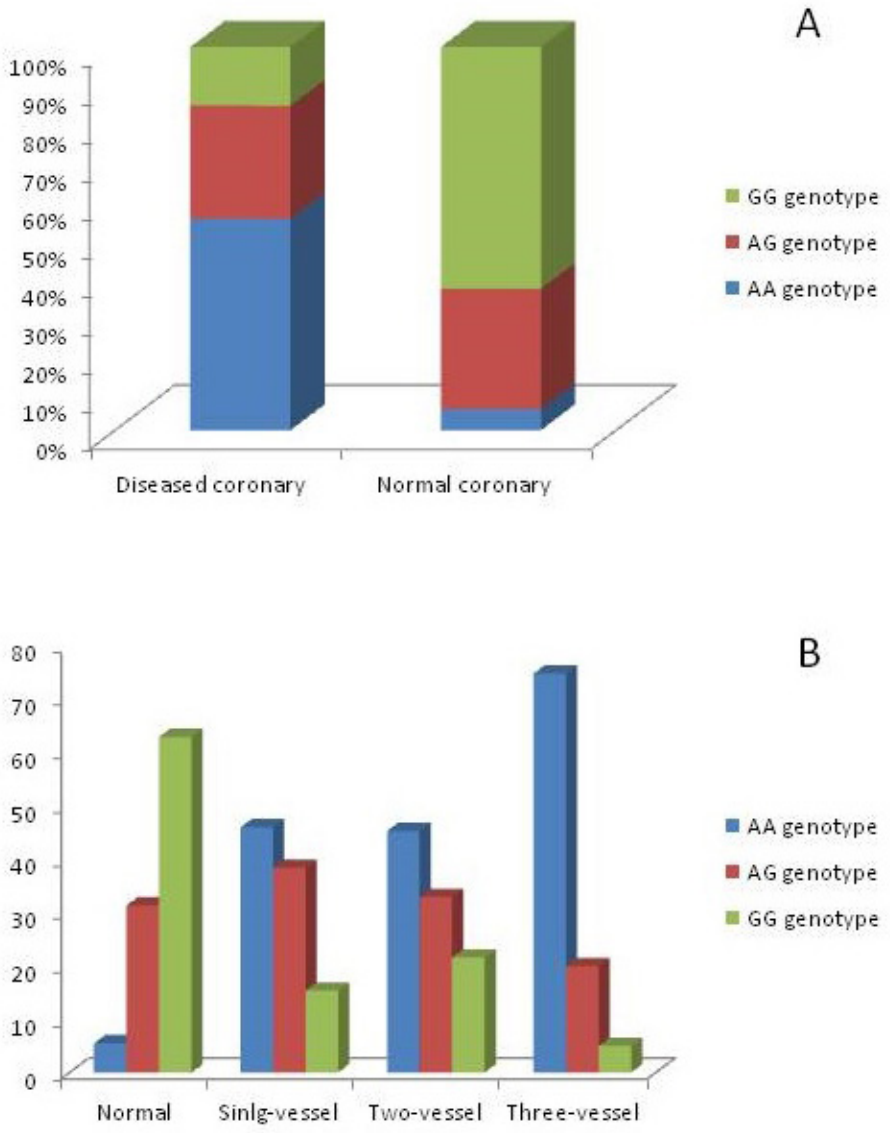

Figure 2. The frequency of *MMP-9* gene genotypes According to the presence of coronary artery disease (A) and its severity (B)

Table 2. The frequency of SNP genotypes and alleles according to coronary arteries states

Item	Normal coronary arteries (N = 471)	Single-vessel disease (n = 26)	Two-vessel disease (n = 97)	Three-vessel disease (n = 60)	P-value
MMP-9 **(rs17576) A/G**					<0.001
AA	26 (5.5)*	12 (46.1)	44 (45.4)	45 (75.0)	
AG	148 (31.4)	10 (38.5)	32 (33.0)	12 (20.0)	
GG	297 (63.1)	4 (15.4)	21 (21.6)	3 (5.0)	
Allele frequency					<0.001
A	200 (21.2)	34 (65.4)	120 (61.8)	102 (85.0)	
G	742 (78.8)	18 (34.6)	74 (38.2)	18 (15.0)	

* The results were indicated as number (percentage)

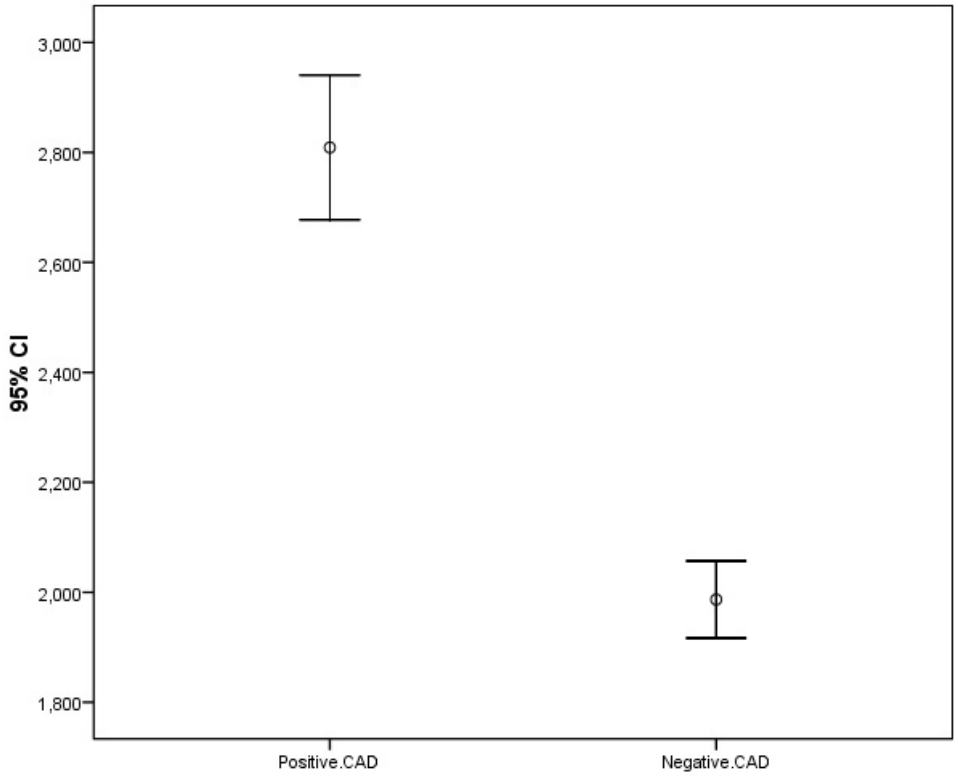

Figure 3. The serum value of MMP-9 in subgroups with and without CAD

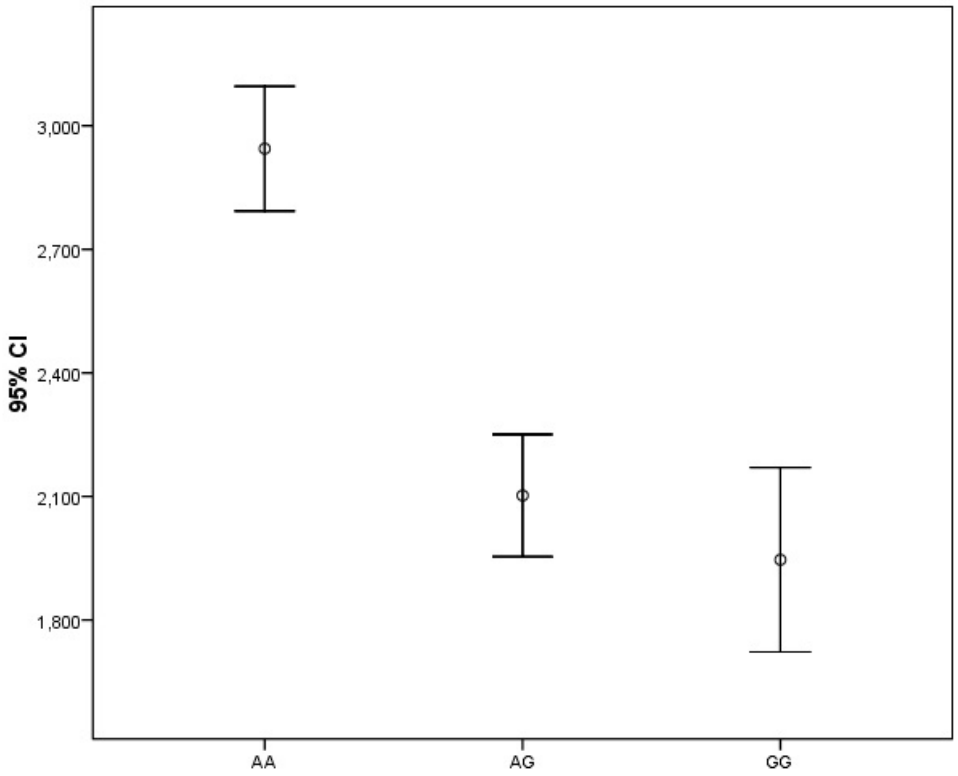

Figure 4. The serum value of MMP-9 according to the genotypes of MMP-9 polymorphism

Table 3. The frequency of SNP genotypes and alleles according to outcomes in CAD group

Item	Mortality		P value	MACE		P-value
	Positive (n = 22)	Negative (n = 161)		Positive (n = 38)	Negative (n = 145)	
MMP-9 **(rs17576) A/G**			<0.001			0.224
AA	17 (77.3)*	41 (25.5)		18 (47.4)	88 (60.7)	
AG	4 (18.2)	22 (13.7)		8 (21.1)	22 (15.2)	
GG	1 (4.5)	98 (60.8)		12 (31.5)	35 (24.1)	
Allele frequency			<0.001			0.636
A	38 (86.4)	104 (32.3)		44 (57.9)	198 (68.3)	
G	6 (13.6)	218 (67.7)		32 (42.1)	92 (31.7)	

* The results were indicated as number (percentage)

Table 4. The Cox proportional hazard analysis to determine the role of dominant allele of rs17576 for predicting one-year death

Item	Beta	Standard Error	P-value	Hazard Ratio (HR)	95% CI for HR	
					Lower	Upper
A versus G allele	1.752	0.635	0.006	5.764	1.659	20.021
Sex	-0.065	0.649	0.920	0.937	0.263	3.342
Age	0.028	0.029	00333	1.029	0.971	1.089
Body mass index	0.402	0.558	0.471	1.495	0.501	4.461
Renal failure	-0.007	0.007	0.283	0.993	0.979	1.006
Multi-vessel dis.	2.232	0.999	0.026	9.345	1.314	36.667
Family history of CAD	-1.411	0.977	0.149	0.244	0.036	1.655
Hyperlipidemia	-1.263	0.760	0.097	0.283	0.064	1.255
Diabetes mellitus	1.886	0.927	0.042	6.172	1.017	40.166
Hypertension	2.008	0.929	0.031	7.462	1.205	28.456
Smoking	0.313	0.972	0.748	1.367	0.203	9.185
Brain stroke	-0.333	1.174	0.777	0.717	0.072	7.164
Baseline LVEF	0.111	0.312	0.721	1.118	0.607	2.059

Discussion

Various risk factors, including molecular and clinical factors, have been identified for the severity of coronary heart disease as well as its short- and long-term consequences. In this regard, the role of gene polymorphisms and changes in the serum levels of specific biomarkers related to these gene polymorphisms have been shown to contribute to poorer outcomes in ischemic heart disease.

Additionally, enzymatic dysfunction resulting from gene polymorphisms—linked to inflammatory processes, oxidative stress, proliferation or apoptosis of vascular smooth muscle cells, endothelial dysfunction, platelet aggregation, and atherosclerotic plaque rupture has been investigated in some studies[17-19]. *MMP-9* plays a major role in various cellular processes related to tissue remodeling, such as cardiac tissue development, neovascularization through proteolytic degradation of proteins in the basal lamina of blood vessels, activation of vascular endothelial growth factors, and regulation of immune cell functions[21].

Given the role of these processes in the formation and progression of atherosclerotic plaques, it is hypothesized that polymorphisms in specific genes related to coronary atherosclerosis and consequently, abnormal changes in serum levels of enzymes encoded by these genes may contribute to the exacerbation of ischemic heart disease. In the present study, this hypothesis was tested to determine whether the presence of certain SNPs related to the *MMP-9* gene and subsequent changes in serum levels of its product indicate a higher

hazard of more severe coronary heart disease as well as poorer long-term outcomes.

Our study clearly showed that, first, the presence of the rs17576 polymorphism (dominant homozygous genotype) was associated with more severe coronary artery disease. Additionally, the serum level of this marker was significantly higher in patients with multi-vessel coronary involvement due to the presence of the studied polymorphism. Therefore, as a primary result, tracking the rs17576 polymorphism can predict the severity of coronary involvement in these patients.

On the other hand, the presence of the corresponding polymorphism was also related to the one-year mortality of these patients, and in fact, this polymorphism could serve as a predictive factor for one-year mortality. However, the presence of this polymorphism did not have a significant relationship with the occurrence of one-year myocardial infarction or cerebrovascular events.

Studies on the role of *MMP-9*-related polymorphisms in predicting ischemic heart involvement have been extensive, but few have specifically investigated the role of rs17576. In a study by Wu et al.[23], although the frequency of variant rs17576 genotypes was similar in the two groups with and without coronary involvement, the serum level of *MMP-9* was significantly higher in the former group. However, in another study by Pogorielova et al.[25], a weak link was observed between *MMP-9* concentration and the presence of acute coronary syndrome. Additionally, their study found no statistically significant association between *MMP-9* serum concentration and myocardial infarction occurrence.

Some studies have correlated the rs17576 polymorphism with plaque formation in carotid arteries[26], while another study linked this polymorphism with myocardial infarction occurrence[26]. Based on a comparison of our results with other studies, the role of the rs17576 polymorphism of *MMP-9* in the development of coronary heart disease in our population appears to be more prominent than in other populations. Genetic and racial characteristics likely exert unique effects on the occurrence of heart diseases across different populations.

Therefore, this polymorphism may have a strong link with the severity of coronary heart disease in the selected Turkish population. It may be specific to the ethnic group, making its use as a predictive factor for coronary disease and severity more evident in this population. Of course, it should be noted that the occurrence of this polymorphism can have a physiopathological effect only when accompanied by changes in serum levels of *MMP-9*, a result that was fully confirmed in the present study.

Nowadays, gene polymorphisms and changes in gene expression have been considered factors for predicting the outcomes of ischemic heart diseases. However, it should be kept in mind that a wide range of clinical, laboratory, and even genomic parameters may be related to the risk of occurrence and exacerbation of ischemic heart diseases, and each of these factors carries a different weight for prediction.

In fact, this study examined only one gene polymorphism, and it is evident that other genetic polymorphisms even with greater predictive strength and weight may also be associated with adverse disease outcomes, which should be explored in future studies. Additionally, it is recommended that the weight of each genomic factor be considered alongside other clinical predictors in the design of scoring systems for outcome prediction.

Despite the valuable results, the study had some limitations. First, the sample size appears to be small for generalizing the findings to the entire Turkish population. Turkey has a high level of racial diversity, and therefore, to achieve more reliable results, it is necessary to include populations of various ethnic backgrounds. Second, the study results would have greater statistical power with a longer follow-up period, so an extended patient follow-up is recommended.

Conclusion

It can be finally concluded that the presence of *MMP-9*-related polymorphism of rs17576

was associated with increasing the hazard of severe coronary artery disease among Turkish population. Tracking this polymorphism can be also effectively used to predict one-year death in such patients and thus can be considered as a major key for setting up the new cardiovascular high-risk scoring systems. Future studies employing Mendelian Randomization tests can further estimate quantitatively the impact of this SNP on CAD.

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Conflict of interests
The authors declare no conflict of interest.

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Author's Contributions
Study conception and Design: MS
Data Acquisition: HD; MAT
Data analysis or Interpretation: HD; SA
Manuscript Drafting: MS
Critical Manuscript Revision: MAT
All authors have approved the final manuscript and are responsible for all aspects of the work.

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