Effects of *MPO-463G/A* and *-129G/A* polymorphisms on coronary artery disease risk and patient survival in a Turkish population

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Abstract. Myeloperoxidase (MPO) is an oxidative hemoprotein compound expressed in polymorphonuclear leukocytes that contributes to inflammatory responses. Coronary artery disease (CAD), as the most prevalent form of heart disease, is considered to originate from an interaction between genetic and environmental factors. In the present study, the potential associations between *MPO-463G/A* and *-129G/A* polymorphisms with CAD were investigated in a Turkish population using a polymerase chain reaction-based restriction fragment length polymorphism (RFLP) assay technique. To the best of our knowledge, the study was the first to examine the association of *MPO-463G/A* and *-129G/A* with patient survival rate in a Turkish population. The study population consisted of 201 patients with CAD and 201 healthy controls. The results indicated that there was a significant association of the *GA* genotype of *MPO-463G/A* with the case population (P=0.048). Meanwhile, in the patients with CAD, the frequency distributions of the *MPO-129A* allele (P=0.006) and *GA* genotype (P=0.001) were significantly increased compared with the *G* allele and *GG* genotype, respectively, in CAD patients. Additionally, compared with the *GG* genotype, the frequency distribution of *MPO-129A* was significantly increased in the patient group regarding smoking status (P=0.001) and the presence of hypercholesterolemia (P=0.028). However, survival analysis did not detect an effect of either polymorphism on the survival rate of the CAD patients (P>0.05). Therefore, the *MPO-129GA* genotype may be a significant risk factor for the development of CAD.

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Introduction

Coronary artery disease (CAD) is established as a major cause of mortality worldwide (1). Pathogenesis of CAD involves the formation of atherosclerotic plaques, which consist of endothelial cells, leukocytes, inflamed smooth muscle cells, necrotic cores, accumulated modified lipids and calcified regions, all of which indicate that CAD is an inflammatory disease in which immune mechanisms interact with metabolic risk factors (2). Polymorphonuclear neutrophils may modulate and signal in inflammatory pathways through the secretion of enzymes including myeloperoxidase (MPO) (3). To date, findings have implicated MPO as a prominent participant in the link between inflammation and cardiovascular diseases such as CAD (4).

MPO is an enzyme expressed by leukocytes, particularly neutrophils and monocytes, that catalyzes the formation of numerous reactive oxidant species at sites of inflammation (5). The human *MPO* gene is located on chromosome 17q23.1 and consists of 11 introns and 12 exons (5). A common single nucleotide polymorphism (SNP) of *MPO* is *MPO-463G/A*, which consists of a substitution from G to A at position 463 bp. Meanwhile, *MPO-129G/A*, as another SNP, is located in the *MPO* gene promoter. Both of these SNPs have been reported to affect the binding of the transcription factor specificity protein 1, and thus, the protein expression of MPO (6,7).

Under physiological conditions, MPO-derived oxidation products serve an important role in host defense, though continuous activation of MPO results in increased levels of reactive chlorine species, and MPO-derived oxidants have been linked with atherosclerosis (8,9). However, whether MPO levels may serve as a marker of plaque vulnerability in the assessment of cardiovascular risk remains uncertain. Therefore, the present study aimed to investigate the association between the *MPO* SNPs *-463G/A* and *-129G/A* and CAD risk in a Turkish population. Additionally, the effect of these SNPs on patient survival rate was examined, and an evaluation of the possible relationships between the SNPs and demographic parameters including sex, age and rates of hypertension, diabetes and hypercholesterolemia, was a further study aim.

Table I. Demographic and clinical parameters of CAD patients and healthy controls.

Variable	CAD cases, n (%)	Controls, n (%)	OR (95% CI)	P-value
Total	201 (100)	201 (100)		
Age, mean ± SD	61.06±6.81	59.87±7.14		0.088
Sex				
Female	86 (42.79)	103 (51.24)		
Male	115 (57.21)	98 (48.76)	1.40 (0.94-2.04)	0.089
Smoking status				
Non smoker	92 (45.77)	110 (54.73)		
Smoker	109 (52.23)	91 (45.27)	1.43 (0.96-2.12)	0.073
Hypertension >130-140/80-90 mmHg				
Absent	73 (36.32)	150 (74.63)		
Present	128 (63.68)	51 (25.37)	5.15 (3.36-7.91)	<0.001
Diabetes				
Absent	124 (61.69)	130 (64.68)		
Present	77 (38.31)	71 (35.32)	1.13 (0.75-1.70)	0.535
Hypercholesterolemia ≥240 mg/dl				
Absent	129 (64.18)	146 (72.64)		
Present	72 (35.82)	55 (27.36)	1.48 (0.97-2.26)	0.068

CAD, coronary artery disease; SD, standard deviation; OR, odds ratio; CI, confidence interval.

Patients and methods

Study population. The study group consisted of 201 patients with CAD (case group; age, 61.06±6.81; 115 male, 86 female) and 201 healthy individuals (control group; age, 59.87±7.14; 98 male, 103 female) enrolled from Cumhuriyet University Hospital (Sivas, Turkey) between June 2011 and December 2011. In all patients with CAD, coronary angiography identified >50% stenosis in at least one major coronary vessel as a result of atherosclerosis. The control group consisted of healthy individuals with a negative family history of CAD. To select the control population, findings of routine clinical tests, physical and laboratory examinations and electrocardiography and echocardiography were evaluated; individuals with negative test results indicative of the absence of pathology were chosen. Participants in the case and control groups were born in Turkey. Information concerning sex, age, the presence of hypertension (>130-140/80-90 mmHg) (10), diabetes and/or hypercholesterolemia (≥240 mg/dl) (11) and smoking habits was collected using a standardized questionnaire. Informed consent was obtained from the patients prior to the study, and the study was approved by the Ethics Committee of the Medical School of Cumhuriyet University (approval no. 2011-02/04).

Genotyping. Genomic DNA of the case and control groups was extracted from blood leukocytes (in 1 ml blood samples) in collection tubes with EDTA using a standard phenol-chloroform method (12). *MPO* genotypes were determined using a polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) assay. The PCR-RFLP was performed according to the method reported by Arslan *et al* (13). The primers for *MPO-463G/A* were forward, 5'-CGGTATAGGCACACAATGGTGAG-3' and reverse, 5'-CAATGGTTCAAGCGATTCTTC-3'; and for *MPO-129G/A* were forward, 5'-CCTCCACAGCTCACCTGATAT-3' and reverse, 5'-CGCTTGAACCATTGCACATCA-3'. The *MPO-463* and *-129G/A* SNP amplicon sizes were 350 and 278 bp, respectively. The PCR products were digested with *Ssi*I (for *MPO-463G/A*) and *Apa*I (for *MPO-129G/A*), according to the manufacturer's instructions (Fermentas; Thermo Fisher Scientific, Inc., Waltham, CA, USA), and fragment sizes were determined by 3% agarose gel electrophoresis and ethidium bromide staining, using a UV transilluminator for visualization. The *-463G/A* genotypes following *Ssi*I digestion were *GG* (169, 120 and 61 bp), *GA* (289, 169, 120 and 61 bp) and *AA* (289 and 61 bp). The *-129G/A* genotypes following *Apa*I digestion were *GG* (278 bp), *GA* (278, 154 and 124 bp) and *AA* (154 and 124 bp). To confirm the MPO genotypes, 15 samples of each genotype (homozygous wild-type, heterozygous and homozygous mutation) were selected for detection using an ABI 310 DNA sequencing system (Applied Biosystems; Thermo Fisher Scientific, Inc.), which was performed externally by Life Technologies, Ltd. (Thermo Fisher Scientific, Inc.).

Statistical analysis. Data are representative of three independent repeat experiments. All statistical analyses were performed using SPSS software version 17.0 (SPSS, Inc., Chicago, IL, USA). The statistical significance of differences in the *MPO* genotypes between the case and control groups were evaluated using χ^2 tests. Multivariate logistic regression analyses were performed for comparisons regarding demographic and clinical parameters (sex, age, presence of hypertension, diabetes and/or hypercholesterolemia and smoking habits), for

Table II. Risk estimates and frequency distributions of alleles and genotypes of *MPO* polymorphisms (*-463G/A* and *-129G/A*) in association with demographic and clinical parameters.

	MPO-463			*MPO-129*		
Variable	Cases/controls, n (%)	OR (95% CI)	P-value	Cases/controls, n (%)	OR (95% CI)	P-value
Total						
G	276 (68.65)/296 (73.63)	Ref.		292 (72.63)/325 (80.84)	Ref.	
A	126 (31.34)/106 (26.36)	1.27 (0.93-1.73)	0.120	110 (27.36)/77 (19.15)	1.59 (1.14-2.21)	0.006
GG	97 (48.25)/116 (57.71)	Ref.	-	91 (45.27)/124 (61.69)	Ref.	
GA	82 (40.79)/64 (31.84)	1.53 (1.00-2.34)	0.048	110 (54.72)/77 (38.30)	1.94 (1.30-2.89)	0.001
AA	22 (10.94)/21 (10.44)	1.25 (0.65-2.41)	0.500	Undetected	-	-
Female						
GG	48 (55.81)/53 (51.45)	Ref.		44 (51.16)/70 (67.96)	Ref.	
GA	30 (34.88)/36 (34.95)	0.92 (0.49-1.71)	0.793	42 (48.83)/33 (32.03)	2.02 (1.12-3.65)	0.019
AA	8 (9.3)/14 (13.59)	0.63 (0.24-1.63)	0.341	-	-	-
Male						
GG	52 (44.44)/63 (64.28)	Ref.		48 (41.73)/55 (56.12)	Ref.	
GA	50 (42.73)/29 (29.59)	2.08 (1.16-3.75)	0.013	67 (58.26)/43 (43.87)	1.78 (1.03-3.07)	0.036
AA	15 (12.82)/6 (6.12)	3.02 (1.09-8.36)	0.027	-	-	-
Smoking						
GG	53 (48.18)/51 (56.04)	Ref.		43 (39.44)/58 (63.73)	Ref.	
GA	42 (38.18)/30 (32.96)	1.34 (0.73-2.47)	0.335	66 (60.55)/33 (36.26)	2.69 (1.51-4.79)	0.001
AA	14 (12.72)/10 (10.98)	1.34 (0.54-3.30)	0.515	-	-	-
Hypertension						
GG	74 (57.81)/24 (47.05)	Ref.		58 (45.31)/29 (56.86)	Ref.	
GA	43 (33.59)/21 (41.17)	0.66 (1.33-1.33)	0.248	70 (54.68)/22 (43.13)	1.59 (0.82-3.06)	0.163
AA	11 (8.59)/6 (11.76)	0.59 (0.19-1.77)	0.349	-	-	-
Diabetes						
GG	45 (58.44)/45 (63.38)	Ref.		37 (48.05)/40 (56.33)	Ref.	
GA	25 (32.46)/21 (29.57)	1.19 (0.58-2.42)	0.631	40 (51.94)/31 (43.66)	1.39 (0.73-2.66)	0.313
AA	7 (9.09)/5 (7.04)	1.40 (0.41-4.74)	0.588	-	-	-
Hypercholesterolemia						
GG	32 (44.44)/29 (52.72)	Ref.		29 (40.27)/33 (51.56)	Ref.	
GA	30 (41.66)/20 (36.36)	1.35 (0.63-2.89)	0.426	43 (59.72)/31 (48.43)	2.22 (1.08-4.55)	0.028
AA	10 (13.88)/6 (10.90)	1.51 (0.48-4.67)	0.473	-	-	-
Haplotype						
MPO						
-463 *-129*						
G *G*	114 (56.71)/132 (65.67)	Ref.				
G *A*	30 (14.92)/21 (10.44)	1.65 (0.89-3.04)	0.105			
A *G*	32 (15.92)/30 (14.92)	1.23 (0.70-2.15)	0.458			
A *A*	25 (12.43)/18 (8.95)	1.60 (0.83-3.09)	0.153			

MPO, myeloperoxidase; OR, odds ratio; CI, confidence interval.

which odds ratios (ORs) and 95% confidence intervals were calculated. Kaplan-Meier analysis was used to compare the survival curves of the subjects determined from a 20-week follow-up. In all cases, P<0.05 was considered to indicate statistical significance.

Results

Demographic and clinical parameters. The present study involved 201 patients with CAD (115 male, 86 female) and 201 healthy controls (98 male, 103 female). The demographic and clinical parameters of the CAD and control populations are summarized in Table I. There was a significantly higher frequency of hypertension in the CAD population compared with the control group (OR=5.15, P<0.001). However, no significant differences were observed between the patients and controls regarding sex distribution, smoking status and the presence of diabetes or hypercholesterolemia (P>0.05).

Allele and genotype frequencies. The allele and genotype distributions of *MPO-463G/A* and *-129G/A* in the CAD cases and controls are presented in Table II. The *MPO-463GG*, *GA* and *AA* genotype frequencies were 48.25, 40.79 and 10.94%, respectively, in the patients with CAD; and 57.71, 31.84

Table III. Risk estimates and frequency distributions of MPO polymorphisms adjusted for age, sex, smoking habit, hypertension, diabetes and hypercholesterolemia.

	MPO-463G/A		*MPO-129G/A*	
Variable	Adjusted OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
Genotype				
GA	1.13 (0.59-2.18)	0.695	1.23 (0.69-2.18)	0.478
AA	1.03 (0.40-2.67)	0.937		
Age	1.07 (1.05-1.10)	<0.001	1.07 (1.05-1.09)	<0.001
Sex	3.69 (1.83-7.44)	<0.001	3.43 (1.75-6.70)	0.001
Smoking	0.50 (0.25-1.00)	0.051	0.48 (0.25-0.92)	0.028
Hypertension	8.38 (4.38-16.05)	0.001	9.78 (5.26-18.21)	0.001
Diabetes	0.47 (0.24-0.90)	0.024	0.58 (0.31-1.07)	0.084
Hypercholesterolemia	0.85 (0.43-1.67)	0.645	0.90 (0.47-1.72)	0.767

Adjusted ORs are relative to the *GG* genotype (reference). The mean ORs of the *GA* and *AA* genotypes are presented for age, sex, smoking, hypertension, diabetes and hypercholesterolemia. MPO, myeloperoxidase; OR, odds ratio; CI, confidence interval.

and 10.44%, respectively, in the healthy controls. The *MPO-463A* allele was identified in 31.34% of CAD patients and 26.36% of controls. Comparing the genotype frequency distributions of *MPO-463GG* and *MPO-463GA* revealed that patients with *MPO-463GA* had a significantly higher risk of developing CAD (OR=1.53, P=0.048). Meanwhile, no significant differences were observed regarding disease risk of the *AA* genotype and *A* allele frequencies compared with the *GG* genotype (OR=1.25, P=0.500) and *G* allele (OR=1.27, P=0.120) frequencies, respectively.

The *MPO-129GG*, *GA*, *AA* genotype frequencies were 45.27, 54.72 and 0.00%, respectively, in the patients with CAD; and 61.69, 38.30 and 0.00%, respectively, in the healthy controls. The *MPO-129A* allele was identified in 27.36% of patients and 19.15% of controls (Table II), and comparing the *A* and *G* allele distributions indicated that *-129A* conferred a significantly greater risk of developing CAD (OR=1.59, P=0.006). Additionally, comparing the *GG* and *GA* genotype distributions revealed that the *GA* genotype was a significant risk factor of CAD (OR=1.94, P=0.001).

On analysis of genotype distribution regarding patient characteristics, *MPO-463AA* was identified to be a significant risk factor in males compared with male *GG* carriers (OR=3.02, P=0.027; Table II). It was also indicated that male patients with the *MPO-463GA* genotype had a significantly higher risk of CAD compared with male *GG* carriers (OR=2.08, P=0.013). With regard to hypertension, *MPO-463G/A* was not a significant risk factor as *GA* (OR=0.66, P=0.248) or *AA* (OR=0.59, P=0.349). For *MPO-129G/A*, the *GA* genotype was a significant risk factor regarding sex (female: OR=2.02, P=0.019; male: OR=1.78, P=0.036), smoking status (OR=2.69, P=0.001) and the presence of hypercholesterolemia (OR=2.22, P=0.028), but not regarding the presence of hypertension (OR=1.59, P=0.163) or diabetes (OR=1.39, P=0.313; Table II).

Haplotype and survival analysis. The haplotypes for all probable haplotypes were examined. All of the four possible haplotypes determined for the two SNPs were observed in the study samples. As presented in Table II, the differences between the frequency distributions of all possible haplotypes did not differ significantly (P>0.05). Furthermore, on Kaplan-Meier analysis to compare the survival curves of subjects with regard to *MPO-463G/A* and *MPO-129G/A*, it was determined that the SNPs had no significant effect on the survival of patients with CAD (for *MPO-463G/A*, P=0.516 and for *MPO-129G/A*, P=0.220; data not shown).

Additionally, the risk estimates and frequency distributions of the *MPO* polymorphisms were adjusted for age, sex, smoking status and the presence of hypertension, diabetes and hypercholesterolemia (Table III). There was a marked association between *MPO-463G/A* and CAD risk on adjustment for hypertension (adjusted OR=8.38), which was deemed to be significant to P=0.001. Furthermore, patients with hypertension exhibited a marked association between *MPO-129G/A* and disease risk (adjusted OR=9.78) significant to P=0.001.

Discussion

CAD is a leading cause of mortality worldwide (14), and results from interactions between numerous genes and environmental factors (15). It is considered that oxidative stress serves a key role in the initiation and progression of atherosclerosis (16). In particular, MPO has been identified to influence the incidence of CAD, and polymorphisms in genes such as MPO are potential modifiers of individual predisposition to CAD (17).

The present study aimed to research the association between CAD and two *MPO* SNPs, *-463G/A* and *-129G/A*, in a Turkish population. For this, the allele and genotype distribution frequencies for *MPO-463G/A* and *-129G/A* were determined in the study population. These data were also investigated in association with the demographic and clinical parameters of the subjects. Previous studies have indicated that *MPO-463A* allele frequency in cases and controls varied from 8 to 47% and 16 to 56%, respectively, in different ethnic populations (18-21). However, it has been reported that *MPO-463A* frequency in control subjects was 26.7% in French-Canadian (15), 22.4%

in Swedish (19) and 43.5% in Turkish (20) populations. In the present study, it was identified in 31.34% of CAD patients and 26.36% of controls. According to a previous meta-analysis (21), the *MPO-463AA* and *GA* genotypes were associated with a 63 and 27% decreased risk of CAD, respectively, relative to the *GG* genotype. However, in the current study, the distributions of the *MPO-463G/A* allele and genotypes did not differ significantly between the CAD and control populations except for *GA*.

MPO is an important factor of the innate immune response, and forms diffusible oxidative substances with antimicrobial activity, though also promotes oxidative damage of host tissues at sites of inflammation (22). This may lead to endothelial dysfunction and unstable plaque formation, which potentially impacts on atherosclerosis formation (23). Previous studies have indicated that MPO may be used as a marker of inflammation in the coronary artery and after myocardial infarction (24,25). Additionally, high plasma levels of MPO have been correlated with cardiovascular events (26). Notably, MPO levels were significantly increased in CAD, though there was no effect of *MPO-463G/A* polymorphism on MPO levels (20). Conversely, according to a another clinical study (4), *MPO-463G/A* lead to a decrease in MPO expression in the genotype *AA*, intermediate levels in *GA*, and higher levels of intracellular MPO in the genotype *GG*. Furthermore, serum cholesterol levels and smoking have been reported as contributing factors in the upregulation of MPO enzymes (20). However, for *MPO-463G/A* in the present study, the majority of demographic and clinical parameters did not differ significantly between the case and control populations; only male individuals with the *GA* and *AA* genotypes were implicated to have a higher risk of CAD. Accordingly, previous study indicated that males developed CAD more frequently than females (27).

CAD susceptibility may be modulated by polymorphisms in oxidative enzymes such as MPO (28). For *MPO-129G/A* in the present study, regarding *A* allele and *GA* genotype frequency distributions, individuals with the *GA* genotype had ~2-fold higher risk of developing CAD than those carrying *GG* (OR=1.94). In a study in 2016 (28), no significant association was observed between *-129G/A* and CAD in an Indian population. Additionally, in the same study, haplotype analysis revealed that *-463G/A* of the *AA* genotype and *-129G/A* of the *GG* genotype significant improved the clinical condition of disease; however, this relationship was not supported by haplotype analysis in the present study. In a Swedish population, the *A* allele of the *MPO-129G/A* promoter polymorphism may serve a protective role against myocardial infarction in women (19). In the current study, both females and males with MPO-129*GA* were implicated to be at significantly higher risk of CAD. Therefore, the *MPO-129G/A* genotype may be associated with upregulation of MPO expression.

Associations of *-463G/A* and *-129G/A* polymorphisms with CAD have previously been reported (28); however, results vary depending on the ethnicity of the study population. Therefore, the current study evaluated the association between these polymorphisms of the *MPO* gene with CAD risk in a Turkish population. Additionally, to the best of our knowledge, survival curves of the patients were analyzed for the first time; however, the survival rates of subjects with or without the SNPs did not differ significantly. As sex, smoking status, and the presence of hypertension, diabetes and/or hypercholesterolemia influence CAD and MPO levels (29), these factors were adjusted for each SNP.

In conclusion, the *MPO-129A* allele was implicated as a risk factor for CAD in the present study. Additionally, significant associations were identified in smoking individuals and in subjects with hypertension between *MPO-129G/A* and CAD risk. Indeed, hypertension and smoking have previously been implicated as independent predictors for CAD (28). Meanwhile, in the total cohort, *MPO-463G/A* was not significantly associated with CAD; however, individuals with hypertension carrying *MPO-463G/A* had a markedly greater risk of developing CAD. Further comprehensive studies are now required to determine whether *MPO-129A* is a marker of CAD.

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