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Association of *tumor necrosis factor-α -308 G/A* gene polymorphism with coronary artery diseases: An evidence-based study

Elaheh Kazemi[1,2,*] | Khadijeh Jamialahmadi[3,4,*] | Amir Avan[5] | Seyed Reza Mirhafez[6] | Javad Mohiti[1] | Maryam Pirhoushiaran[2] | Nedasadat Hosseini[2] | Akram Mohammadi[2] | Gordon A. Ferns[7] | Alireza Pasdar[2,8] | Majid Ghayour-Mobarhan[5] iD

[1]Department of Biochemistry, International Campus of Shahid Sadoughi University of Medical Sciences, Yazd, Iran

[2]Molecular Medicine Group, Department of Modern Sciences and Technologies, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[3]Biotechnology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

[4]Department of Medical Biotechnology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[5]Metabolic syndrome Research center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[6]Department of Basic Medical Sciences, Neyshabur University of Medical Sciences, Neyshabur, Iran

[7]Division of Medical Education, Brighton & Sussex Medical School, Brighton, Sussex, UK

[8]Division of Applied Medicine, Medical School, University of Aberdeen, Aberdeen, UK

Correspondence
Majid Ghayour-Mobarhan, Biochemistry of Nutrition Research Center, Faculty of Medicine, Mashhad University of Medical Science, Mashhad, Iran.
Email: ghayourm@mums.ac.ir
Alireza Pasdar, Department of Modern Sciences & Technologies, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran and Division of Applied Medicine, Medical School, University of Aberdeen, Foresterhill, Aberdeen, UK.
Emails: PasdarA@mums.ac.ir; a.pasdar@abdn.ac.uk

*Equally contributed as first author.

Background: Coronary artery disease (CAD) is the leading cause of death worldwide and remains a major health problem, providing the rationale for identification of molecular markers for detection of individuals at high risk of developing CAD. Tumor necrosis factor-α (TNF-α) plays a crucial role in the pathogenesis of CAD. We have therefore explored the association of *TNF-α 308 (G/A)* gene polymorphism in 903 individuals with/without CAD.

Methods: *TNF-α 308* gene polymorphism was analyzed in 903 subjects of whom 222 were healthy controls. Among the 681 patients who were investigated angiographically, 468 had ≥50% stenosis and 213 patients had <50% stenosis. Biochemical profiles (eg, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, fasting blood glucose, and CRP) were evaluated. Associations between TNF-α genotypes with biochemical and anthropometric characteristics were determined.

Results: The frequencies of *TNF-α-AA or AG* genotypes were significantly lower in patients classified as CAD patients with ≥ or <50% obstruction in at least one coronary artery, compared to the control group. We observed that CAD patients with ≥50% stenosis and with AA genotype were associated with higher risk of CAD with OR of 3.56 (95%CI: 1.02-12.41; *P*=.046) using multivariate analysis. Moreover, we found that *TNF-α-308-AA* genotype was associated with blood pressure and CRP level in CAD patients, compared to the wild type-genotype.

Conclusion: Our data showed an association of *TNF-α-308G/A* polymorphism with CAD patients with ≥50% obstruction, supporting the need for further investigations on the role of *TNF-α-308G/A* polymorphism with hypertension.

KEYWORDS
coronary artery disease, genotype, tumor necrosis factor-α

1 | INTRODUCTION

Cardiovascular disease, including coronary artery disease (CAD), is the leading cause of death worldwide.[1,2] CAD is a chronic inflammatory process which is mediated, in part, by pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α). It has been shown that TNF-α plays an essential role in the pathogenesis of CAD[3-5] as well as in different processes that may contribute to CAD risk, such as insulin resistance,[6] lipid metabolism disorders, and systemic lupus erythematosus (SLE).[7] The human *TNF-α* gene is located near the major histocompatibility complex (MHC) class III region on chromosome 6p21.3.[8-10] Several functional promoter polymorphisms have been identified in this gene.[11-15]

There is emerging evidence that of an association of *TNF-α-308* polymorphism, a G/A transition at -308-bp position, which may alter promoter activity, with TNF-α expression in vitro, as well as plasma concentrations.[16] An increased serum concentration of TNF-α is an independent predictor of cardiovascular disease [17] and is a marker for recurrent coronary events following a previous acute myocardial infarction.[18] This polymorphism is associated with several conditions,[19-21] including infection, asthma,[20] autoimmune disease,[21,22], and immune-mediated diseases, such as rheumatoid arthritis.[22] The *TNF-α-308* polymorphism is also considered to play a key pathogenic role in CAD.[23]

The relationship between the *TNF-α 308G/A* gene polymorphism (rs1800629) has not been studied in Iranian population with CAD. The aim of our study was to evaluate the association of the *TNF-α 308G/A* polymorphism with the presence of CAD and its association with other important clinical variables in an Iranian population consists of 903 subjects.

2 | MATERIALS AND METHODS

2.1 | Population

In the present cross-sectional study, 903 subjects, including 681 patients with clinically diagnosed CAD who had angiographically defined disease, and 222 controls, without evidence of CAD, all from Mashhad city, in northeastern Iran, were recruited. Patients who had a history of stroke, myocardial infarction, and diabetes mellitus were excluded from this study. Indications for coronary angiography were stable or unstable angina, acute MI, recurrence of symptoms after restoring reperfusion, valvular heart disease, patients undergoing non-cardiac surgery (preoperative), congestive heart failure (CHF), congenital heart disorders, aortic dissection, hypertrophic cardiomyopathy, arteritis, and chest trauma, based on ACC/AHA guidelines for coronary angiography (report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines). According to the results of angiography, the subjects were divided into two groups. Patients whose angiography results showed ≥50% obstruction in at least one coronary artery, who were assigned to the obstructive coronary disease group (n=468). Patients who had less than 50% obstruction in their coronary arteries were assigned to the non-obstructive coronary disease group (n=213). Healthy subjects were those whom referred for their annual medical checkup or pre-employment examinations. They were used as the reference (control) group as they gave no history of cardiac symptoms and their electrocardiogram (ECG) had no abnormalities. The study was performed according to the declaration of Helsinki. Informed consent was obtained from all subjects using protocols approved by the Ethics Committee of the MUMS.

2.2 | Anthropometric measurements

Anthropometric parameters including weight, height, BMI, waist circumference, hip circumference, and waist/hip ratio as well as systolic and diastolic blood pressures were measured as previously described.[24]

2.3 | Lipid profile, fasting glucose, and hsCRP measurements

Total serum cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG), and fasting blood glucose (FBG) concentrations were determined by standard enzymatic techniques, while serum C-reactive protein (CRP) levels were measured using a polyethylene glycol-enhanced immunoturbidimetry method, as described previously.[24]

2.4 | Genotyping

Peripheral blood samples were obtained from the individuals, and genomic DNA was extracted using a Biogene DNA extraction kit according to the manufacturer's protocol (Biogene, Iran). A polymerase chain reaction-amplification refractory mutation system (ARMS-PCR) was performed to determine the *TNF-α gene* polymorphism at position -308 G/A. The two forward and reverse primers used for amplification of *TNF-α* gene were as follows: Forward primers: 5′- ACC CAA ACA CAG GCC TCA GGA CTC AAC A-3 and 5′- TGG AGG CAA TAG CTT TTG AGG GGC AGG A -3′; reverse primers: 5′- AGT TGG GGA CAC GCA AGC ATG AAG GAT A -3′and 5′- TAG GAC CCT GGA GGC TAG ACC CCG TAC C -3′. The reaction was performed in total volume of 20 μL, using 2 μL Buffer, 1.6 μL dNTPs, 2 μL MgCl$_2$, 1 μL for each forward and reverse outer primers, 3 μL for each forward and reverse inner primers, 0.2 μL Taq Polymerase, 20ng DNA, and distilled water. The samples were amplified using the following thermal profile: 94°C for 4 minutes followed by 30 cycles of denaturation at 94°C for 40 seconds, annealing at 62°C for 30 seconds, DNA extension at 72°C for 35 seconds with final extension at 72°C for 5 minutes. PCR system Veriti 96 well thermocycler (Applied Biosystems, USA) was used for amplification. PCR products were then separated by 2% agarose gels for 45 minutes at 80 V, and stained with ethidium bromide.

2.5 | DNA sequencing

DNAs were amplified as described above, sequenced using Sanger sequencing and then analyzed with DNA Analyzer (Sequetech, USA) to confirm the genotypes obtained by ARMS-PCR.

2.6 | Statistical analysis

Data were analyzed using SPSS-11.5 software (SPSS Inc., IL, USA). The normality of distribution was assessed using the Kolmogorov-Smirnov test. Data were expressed as mean±SD (standard deviation) for normally distributed variables or as median±IQR for not normally distributed variables. For normally distributed variables, the student *t* test was used to compare the baseline demographics between the groups. Mann-Whitney *U* test, Chi-square test, and/or Fisher exact test was used for not normally distributed variables. Logistic regression was used to determine the odds ratios for association of the polymorphism and CAD. A two-sided *P*-value <.05 was considered statistically significant.

3 | RESULTS

3.1 | Characteristics of the population

The clinical features and baseline characteristics of the subjects are summarized in Table 1. Not surprisingly, the levels of TG, FBG, CRP, SBP, and DBP were significantly higher in the CAD patients with ≥50% obstruction in at least one coronary artery, compared to the CAD patients with <50% obstruction. Moreover, CAD patients with ≥50% obstruction also had markedly higher levels of TG, HDL-C, CRP and FBG, compared to the other group (Table 1).

3.2 | Association of TNF-α -308G/A polymorphism with baseline characteristics of the groups

In order to explore whether there was an association between *TNF-α -308G/A* polymorphism and clinical and baseline characteristics of CAD patients and control groups, we performed genotyping as described earlier (Figure 1). Genotyping was successfully performed, and no discrepancies were found in the samples analyzed in duplicate. As shown in Table 2, the *TNF-α-AA* genotype had a frequency of 13.2%, whereas the AG and GG genotypes were found in 33.8% and 53% of the CAD patients with ≥50% obstruction, respectively, while these frequencies in the other CAD group were 14.9% (AA), 39.6% (AG), and 45.5% (GG). There was no significant difference in genotype frequencies between the patient and control groups (Table 2).

Furthermore, we explored the association of the genotypes with biochemical and anthropometric characteristics of the populations. This analysis showed that the *TNF-α-308G/A* polymorphism was related to an altered systolic blood pressure and CRP level in CAD patients (Table 3). Individuals with the 308G/A polymorphism-AA genotype or those who carried the A allele of the 308G/A polymorphism were likely to have CAD (Table 4). In particular, AA genotype in CAD patients with ≥50% obstruction was associated with higher risk of CAD with OR of 3.56 (95%CI: 1.02-12.41; *P*-value=.046) (Table 4) using multivariate analysis.

TABLE 1 Demographic, anthropometric, and biochemical characteristics of the population

Variables	CAD patients with ≥50% obstruction (n=468)	CAD patients with <50% obstruction (n=213)	Control (n=222)	*P*-value		
				P1	P2	P3
Age, (y)	57±10	52±10	51±10	<.001	<.001	<.001
Sex, male (%)	281 (63.0)	64 (30.0)	101 (45.4)	<.001	<.001	<.001
BMI (kg/m²)	27±5	26±5	26±4	.1	>.05	>.05
Weight(kg)	70±13	67±14	70±12	.05	.05	.5
WC (cm)	93±12	92±12	92±12	.3	>.05	>.05
Height(cm)	159±19	158±14	144±49	<.001	.8	<.001
TC(mg/dL)	169±45	170±45	194±37	<.001	.5	<.001
TG(mg/dL)	142 (59)	132 (54)	118 (97)	<.001	<.001	.001
HDL-C(mg/dL)	40±13	43±11	41±8	.03	.02	.9
LDL-C(mg/dL)	98±34	99±36	118±36	<.001	.9	<.001
FBG(mg/dL)	131±60	115±49	87±27	<.001	.001	<.001
HC(cm)	99±9	98±8	101±8	.03	.3	.2
SBP(mmHg)	139±25	135±24	123±18	<.001	.2	<.001
DBP(mmHg)	83±11	82±11	76±10	<.001	.2	<.001
MCI (%)	230 (49.1)	103 (48.3)	-	<.001	-	-
hsCRP (mg/dL)	5.5 (2.1-6.4)	4.3 (1.7-4.9)	1.7 (1.1-3.5)	.199	.025	.718

Values are expressed as mean±SD or median range. Comparisons were made by the χ2 test (for categorical data), one-way ANOVA and Kruskal-Wallis test (for numerical data). Also, the post hoc test was used for comparison between groups. P1: comparison between CAD patients with ≥50% obstruction and CAD patients with <50% obstruction group. p2: comparison between CAD patients with ≥50% obstruction and control group. P3: comparison between CAD patients with <50% obstruction and control group. BMI: body mass index; TC: total cholesterol; WC: Waist circumference, HC: Hip circumference, TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; FBG: fasting blood glucose; SBP: systolic blood pressure; DBP: diastolic blood pressure; MCI: History of myocardial infarction.
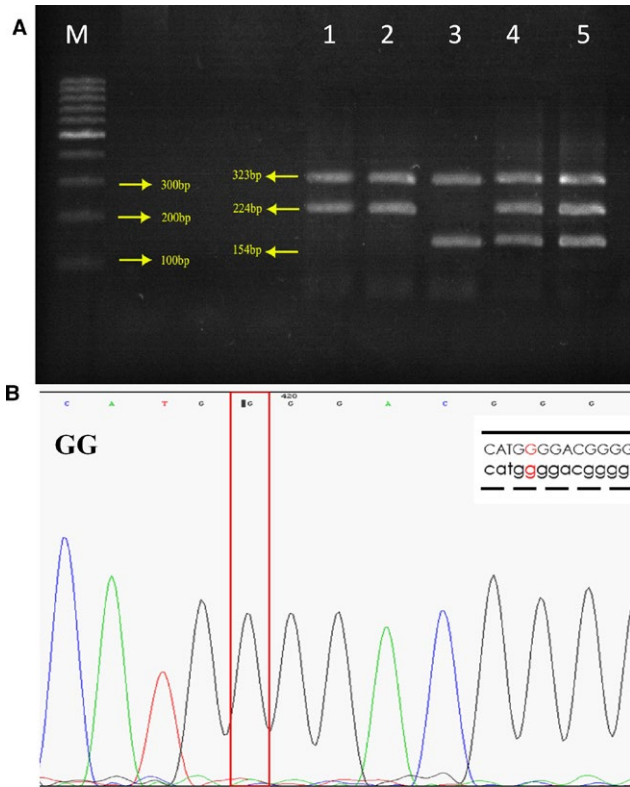
FIGURE 1 (A-B) ARMS-PCR and sequence of the amplified fragment in TNF-α gene polymorphism. The genotype was labeled on corresponding sequences, and the sites which were marked were the SNP of TNF-α gene electrophoresed on 2% agarose, stained with ethidium bromide. Left to right: M, DNA marker (100bp), Lanes 1 and 2: GG homozygous (323bp and 224 bp), Lane 3: homozygote AA (323bp and 154 bp) and Lanes 4 and 5: AG heterozygote (323 bp, 224 bp, and 154 bp) for TNF-α genotype

4 | DISCUSSION

We demonstrated that the AA genotype of TNF-α 308 gene polymorphism was associated with the presence of ≥50% coronary artery stenosis in patients being investigated by angiography.

TNF-α alters vascular endothelial cell function, inducing the production and activation of growth factors and adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin.[25-26] It has been shown that TNF-α plays an important role in the pathogenesis of atherosclerosis. It has been reported that patients with CAD have circulating mononuclear leukocytes that have an increased potential to secrete TNF.[27]

There is growing evidence of an association between the TNF-α SNP and cardiovascular disease.[21] However, the data are inconsistent.[16,27,28] Ghazouani et al.[16] have reported that the TNF-α 308 (G/A) SNP was not associated with CAD in Tunisian patients. A further study also showed the lack of association of TNF-α 308G/A polymorphism and risk of coronary artery disease and myocardial infarction.[27] Koch et al.[29] evaluated the association between this genetic polymorphism and coronary artery disease in a Caucasian population finding no association with CAD. Padovani and colleagues investigated two genetic polymorphisms in the TNF-α gene (-308 G→A and LT-alpha +252 A→G) as risk factors for CAD by determining its prevalence in 148 survivors of myocardial infarction (MI) with angiographically proven severe CAD.[30] They were unable to find a significant association between the TNF-α polymorphisms and MI.[27] This lack of association can be explained at least in part by life style, severity of the disease, small sample size, and ethnic diversity.

In the present study, we investigated the association of TNF-α -308G/A polymorphism in a large group of patients with angiographically defined disease. Our data showed that individuals carrying A allele for TNF-α were at a higher risk of having CAD, which is in line with previous observations. In particular, Kriskovszky and colleagues found that the TNF-α 308A was related with the low blood pressure in diabetic adolescents.[31] Another study showed that TNF-α 308G was associated with increased risk of hypertension.[32] Ito et al.,[33] investigated the correlation of TNF-α with atherogenic risk factors in women. They observed that the serum level of TNF-α was significant related with obesity, hyperlipidemia and hypertension. These findings are in agreement with our recent data about the role of this marker with hypertension, CAD, and metabolic syndrome.[33-37] Moreover, a recent meta-analysis revealed that the A allele of TNF-α G308A gene increases the risk of essential hypertension,[38] which is in agreement with our data.

The strength of the present study is that it was carried out in a large number of individuals with or without angiographically defined CAD. The main limitations of this study were the cross-sectional study design and possible confounding effects of differences in

TABLE 2 Genotype distribution and allele frequency of TNF-α polymorphism in all the subjects

Genotype	CAD patients with ≥50% obstruction, %	CAD patients with <50% obstruction, %	Control n=222, %	Significance
Genotype distribution				
(GG)	53.0 (248)	60.6 (129)	45.5 (101)	χ^2=10.48; df=4; P=.03
(AG)	33.8 (158)	26.8 (57)	39.6 (88)	
(AA)	13.2 (62)	12.7 (27)	14.9 (33)	
HWE	P<.05	P<.05	P=.17	
Allele frequency				
A	30.1 (282)	26.1 (111)	34.7 (154)	χ^2=7.689; df=2; P=0.021
G	69.9 (654)	73.9 (315)	65.3 (290)	

Comparisons were made by the χ^2 test.

TABLE 3 Association of TNF-α polymorphism with biochemical and anthrometric characteristics of subjects

Groups	Genotype	Height	Weight	BMI	WC	HC	TC	TG	HDL	LDL	FBG	SBP	DBP	hsCRP
Control	GG	161±9	69±12	25±4	91±12	99±7	192±37	135.3±70.6	41±8	115±38	84±16	121±16	74±10	1.4 (0.8-2.3)
	GA	159±14	71±13	26±4	92±13	102±10	193±36	133.5±76.2	41±8	120±32	92±38	125±20	77±9	1.7 (1.0-5.0)
	AA	159±9	71±9	26±2	94±10	103±7	203±41	154.2±108.2	39±8	121±43	85±15	119±17	77±12	3.5 (1.3-10.4)
	P	.4	.2	.8	.4	.02	.3	.7	.2	.5	.1	.2	.1	<.001
CAD patients with <50% obstruction	GG	157±16	68±13	27±5	93±11	99±9	171±47	133.4±58.9	43±11	100±38	116±49	134±23	81±11	3.9 (1.6-4.9)
	GA	159±9	66±14	25±5	93±13	100±9	173±44	148.6±99.9	43±10	99±36	110±38	142±25	85±12	3.9 (1.5-4.8)
	AA	156±21	69±14	27±5	94±11	100±9	160±38	126.6±56.9	42±9	97±25	124±69	127±24	81±12	4.8 (3.8-9.1)
	P	.7	.4	.3	.5	.3	.4	.5	.9	.9	.5	.01	.09	.023
CAD patients with ≥50% obstruction	GG	160±20	70±14	27±5	93±11	92±9	172±44	151.9±74.1	40±9	101±36	127±60	140±24	84±10	5.3 (1.7-6.4)
	GA	158±20	70±12	27±5	93±13	100±10	165±43	144.6±75.1	42±19	95±31	136±64	134±25	81±10	5.8 (2.3-6.5)
	AA	160±11	69±13	27±5	93±12	100±9	171±55	171.7±113.2	39±10	94±29	134±53	143±28	80±13	5.4 (2.3-6.8)
	P	.7	.8	.8	.9	.4	.3	.3	.2	.1	.3	.03	.002	.125

BMI, body mass index; WC, Waist circumference; HC, Hip circumference; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FBG, fasting blood glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Values are expressed as mean±SD or median and interquartile range. Comparisons were made by the χ2 test (for categorical data), one-way ANOVA and Kruskal-Wallis test (for numerical data). Also, the post hoc and Mann-whitney test were used for comparison between groups.

P-value <.05 was considered significant.

TABLE 4 Association between TNF-α polymorphism and CAD

	Genotypes	Univariate analysis OR (95% CI)	P	Multivariate analysis OR (95%CI)	P[a]
CAD patients with<50% obstruction	GG	1	-	1	-
	GA	0.507 (0.33-0.77)	.002	0.96 (0.43-2.17)	.968
	AA	0.64 (0.36-1.13)	.127	2.35 (0.64-8.56)	.193
CAD patients with ≥50% obstruction	GG	1	-	1	-
	GA	0.73 (0.51-1.03)	.078	1.66 (0.81-3.40)	.163
	AA	0.76 (0.47-1.23)	.276	3.56 (1.02-12.41)	.046

OR, Odds ratio.

[a]After correction for age, sex, smoking, HDL-C, TG, LDL-C, FBG, SBP, DBP, and BMI.

mean age or gender on the outcome in subjects with CAD patients with <50% obstruction vs CAD patients with ≥50% obstruction in at least one coronary artery, although these variables were partially adjusted for in the logistic regression model and multivariate analysis for evaluating the role of this polymorphisms and CAD. Moreover, we cannot exclude the possible ethnic heterogeneity which might be present in Iranian population, supporting further studies in this population.

In conclusion, we demonstrate the possible important role of *TNF-α-308G/A* polymorphism with blood pressure and its association with CAD. Since accumulating data is supporting the role of *TNF-α* in CAD, further studies are required to investigate the association of other polymorphisms of *TNF-α* with coronary artery disease.

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