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T786C and G894T eNOS polymorphisms as a risk assessment of coronary artery disease

Abstract

Objective: Atherosclerosis is a disease develops due to many reasons in the early stages of life, and after decades results in coronary artery disease. Endothelial dysfunction is an independent factor that helps to predict the future cardiovascular events and occurs before the structural changes of atherosclerosis. The most prominent cause of endothelial dysfunction is nitric oxide reduction. With the determination of the decreased nitric oxide effect on atherosclerosis development, various mutations were detected in the eNOS encoding gene. It has been reported these mutations may lead impaired release of nitric oxide and eventually to coronary artery disease. In this study, we aimed to determine the possible role of eNOS polymorphisms T786C and G894T in coronary artery disease.

Methods: Coronary angiography treated 175 patients in Cardiology Department and Department of Cardiovascular Surgery were grouped as patient group (n=89, 70% or more stenosis in any coronary arteries) and control group (n=86, no stenosis or lesion). Fasting blood sugar, lipid profiles were determined by enzymatic colorimetric method in all individuals. eNOS T786C and G894T polymorphisms were determined by real-time PCR.

Results: In T786C polymorphism of eNOS, compared with the TT genotype, it was determined that those with TC genotype has 2100-fold (p=0.026) and those with the CC genotype has 2.842 times (p=0.040) greater risk of developing coronary artery disease. eNOS G894T polymorphism was shown no significant difference between patient and control group.

Conclusion: The eNOS G894T genotypes were not associated with coronary artery disease, and eNOS T786C variants could be evaluated as a coronary artery disease risk factor.

Keywords: coronary artery disease, eNOS G894C, eNOS T786C, nitric oxide

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Abbreviations: NO, nitric oxide; NOS, nitric oxide synthase; nNOS, neuronal NOS; iNOS, inducible NOS; eNOS, endothelial NOS; CAD, coronary artery disease; FBS, fasting blood sugar; TG, triglyceride; LDL, low density lipoprotein; VLDL, very low density lipoprotein high; HDL, high density lipoprotein

Introduction

Endothelium is a tissue which consists a single row of flat epithelial cells settled on the basement membrane extending between vascular smooth muscle and vascular lumen, that plays role as a barrier involved leukocyte adhesion and materials transvascular diffusion, secretion and the regulation of vasoactive substances, contraction and relaxation of vascular smooth muscle, in regulating of coagulation. Endothel's importance is revealed by the discovery of nitric oxide, an endothelial-derived vasodilator factor, in many diseases. Nitric oxide (NO) is a vasoactive substance which is synthesized from L-arginine by endothelial nitric oxide synthase in the vascular endothelium and is the main mediator of endothelium-dependent vasodilation with vascular smooth muscle relaxant effect.[1]

NO plays an important role in the regulation of cardiovascular physiology, and it has been shown that NO has various effects to inhibit the development of atherosclerosis in the vascular endothelium. Preventing platelet and leukocyte adhesion to the endothelium, decreasing vascular smooth muscle cell migration and proliferation, preventing the transformation of atherogenic LDL by limiting the oxidation of LDL are among the NO various effects aforementioned.[2,3]

Nitric oxide synthase (NOS), which leads the formation of NO, has three isoforms; nNOS (neuronal), iNOS (inducible), and eNOS (endothelial). While the effects of eNOS and nNOS are protective, iNOS may lead to vascular dysfunction and atherosclerosis. Endothelial nitric oxide synthase (eNOS) is the only isoform of NOS that synthesis continually both in vivo and in vitro. eNOS gene is located on terminal region of the chromosome 7, and it is composed approximately 21 kilobases of genomic DNA and contains 26 exons. mRNA encoded by the eNOS gene contains 4052 nucleotides and is located on haploid genome as a single copy.[4,5]

In recent years, several mutations were determined in the eNOS encoding gene. One of the identified mutation in the eNOS gene is displacement of thymidine at nucleotide 786 with the cytosine (T786C), another appears as displacement of guanine at nucleotide 894 with the thymine (G894T). It has been reported that these mutations cause impaired NO release, and may lead to coronary artery disease as a result.[6,7]

Coronary artery disease (CAD) is a disease that results as myocardial ischemia due to decreased blood flow to the heart muscle. Ischemia is a pathological condition that is resulting as tissue damage and usually develops due to insufficient blood reach to a part of the heart such reasons as atherosclerosis, thrombosis, spasm or embolism; or due to reduction in blood flow such reasons as anemia, carboxyhemoglobinemia or hypotension. Despite major advances in the treatment of cardiovascular disease, atherosclerosis ranks first place in the cause of death and it is important in life limiting in men and women in many countries. Atherosclerosis that resulting in coronary artery disease starting from the early stages of life, in middle age and beyond, is related to many reasons.[8]

Smoking, hypercholesterolemia, hypertension, diabetes, age, gender and genetic predisposition are the traditional risk factors in the development of atherosclerosis. Determination of genetic background of coronary artery disease is important to learn earlier whether there is susceptibility to CAD and to take precautions for improving the quality of persons' life. It has been demonstrated in animal experiments that chronic inhibition of eNOS enzyme accelerates atherosclerosis development and causes a rise in blood pressure. It was shown that NO activity was low in coronary arteries of patients with vasospastic angina. These findings are supported the effect of decreased NO release on atherosclerosis development.[1,9]

In recent years, interest of studies especially about the genetic factors that play a role in the pathogenesis of atherosclerosis are increased because of it is one of the most common illness in developed countries. In this study, due to NO's important role in cardiovascular physiology, it was aimed to identify eNOS T786C and G894T gene polymorphisms possible role in patients with CAD, and also to evaluate control group's results of the eNOS T786C and G894T gene polymorphisms in healthy people by finding allele frequency and to determine the prevalence in the community, in Mersin region.

Materials and methods

A total of 175 individuals that presenting to Cardiology and Cardiovascular Surgery clinics with chest pain and coronary artery stenosis of 70% or more results detected with coronary angiography, between the ages of 37 and 83, 89 (18 female and 71 male) CAD (as patient group), and normal coronary angiography results detected with coronary angiography, between the ages of 33 and 88, 86 subjects (38 women and 48 male, as control group) were included in this study. After receiving a confirmation from Ethical Committee (02.03.2011, 2011/41), all individuals participating in the study were informed about the study and written consents were taken.

Patients and control group's demographic characteristics and medical history were collected. Patients were evaluated for risk factors of atherosclerosis such as history of hypertension and diabetes. Patients and control group's peripheral venous blood were taken to tubes containing EDTA and to the biochemistry tubes. DNA was isolated from blood samples in tubes containing EDTA to detect G894T and T786C gene polymorphisms of the eNOS and these polymorphisms were determined by real-time PCR (LightCycler 480 II, Roche Diagnostics GmbH Mannheim, Germany) with mutation detection kits. Biochemistry tubes were centrifugated at 3000rpm for 10minutes and serum were separated. Then fasting blood sugar (FBS), lipid profiles were determined by enzymatic colorimetric method (Cobas Integra 800 autoanalyser, Roche Diagnostics GmbH Mannheim, Germany).

Continuous data (FBS, HDL, LDL, VLDL and TG values) were analyzed by the Shapiro Wilk test whether they were in accordance with normal distribution.

Independent sample t test was used for the comparison of two independent groups in the normal distribution, and Mann Whitney U test was used in the normal distribution. Kruskal-Wallis test was used to compare more than two groups. Chi-square test was applied to analyze the distribution of alleles in genotypes and consistent with the expected value and this distribution (Hardy-Weinberg equilibrium). Possible risks of genotypes and alleles were determined by calculating the odds ratio. Chi-square test was used for comparison of genotypes with other parameters. $p<0.05$ was considered as significant for results.

Results and discussion

A total of 175 individuals, 56 female and 119 male, were included in this study. 89 individuals, who had ≥70% stenosis in major epicardial vessels or branches, identified as patient group (CAD), and normal coronary detected 86 individuals were identified as control group after coronary angiography. Descriptive information and distribution of risk factors for control and CAD group are given in Table 1.

Age values were found significant difference between patients and control group ($p=0.018$). Male patients were significantly higher in the CAD group than the control group ($p=0.001$). Hypertension (HT) was found to be a risk factor in creation of CAD ($p=0.023$). There was no significant relationship between cigarette consumption ($p=0.720$) and diabetes mellitus (DM) ($p=0.652$) variables and CAD.

The distribution of CAD patients were detected according to vessel occlusion; 1 occluded vessel in 29 patients, 2 occluded vessels in 30 patients, 3 and more occluded vessels in 30 patients. FBS and lipid profile values of all individuals who form the control and CAD groups are given in Table 2. When the data were analyzed utilizing the Shapiro Wilk test; except for HDL ($p=0.629$), serum FBS, TC, LDL, VLDL and TG were found that not to show a normal distribution ($p<0.001$). There was no significant differences between patient and control groups ($p> 0.05$) for FBS ($p=0.630$), TC ($p=0.396$), HDL ($p=0.965$), LDL ($p=0.099$), VLDL ($p=0.887$), and TG ($p=0.121$) values.

Consistency between T/C allele genotype belonging to the eNOS T786C variant with to be expected genotypes (Hardy-Weinberg equilibrium) are given in Table 3, in control and CAD groups. The population was found to be in balance for both groups ($p> 0.05$).

The risks posed by genotype and allele, between control and CAD groups, were analysed with binary logistic regression analysis, and eNOS T786C genotype and allele distribution and risks that they create are given in Table 4. In this study three different genotypes, TT, TC and CC, were determined for eNOS T786C. The TT genotype was incorporated by reference in making calculations. The risk of developing CAD was found in TC genotype 2.1-fold (OR=2.100 (1.092-4.051)), in CC genotype 2.842 times (OR=2.842 (1.048-7.708)), and in TC+CC genotype 2.265 times greater (OR=2.265 (1.235-4.153)) when compared to TT genotypes. In addition, the risk of disease was 1.964 times higher in the C allele carrying individuals compared to T allele carrying individuals in eNOS T786C polymorphism ($p=0.005$).

The relationship between the number of obstructed vessels and eNOS T786C genotypes in the CAD group are given in Table 5. There was no significant difference between eNOS T786C TT, TC, CC genotypes and the number of obstructed vessels ($p=0.110$).

Consistency between G/T allele genotype belonging to the eNOS G894T variant with to be expected genotypes (Hardy-Weinberg equilibrium) are given in Table 6, in control and CAD groups. The population was found to be in balance for both groups ($p> 0.05$).

The risks posed by genotype and allele between control and CAD group were analysed with binary logistic regression analysis, and eNOS G894C genotype and allele distribution and risks that they create are given in Table 7. In this study three different genotypes, GG, GT and TT, were determined for eNOS G894T. The GG genotype was incorporated by reference in making calculations. There has not been an interpretation of odds ratio, because $p> 0.05$, and it is meaningless. It was determined that having T allele or TT genotype is not a risk factor for CAD.

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The relationship between the number of obstructed vessels and eNOS G894T genotypes in the CAD group are given in Table 8. There was no significant difference between eNOS G894T GG, GT and TT genotypes and the number of obstructed vessels (p=0,539).

Table 1 Descriptive information and distribution of risk factors for control and CAD group

		Control	CAD	p
Age		56.6±12.9	60.9±10.9	0.018
		n (%)	n (%)	
Gender	Female	38 (44.2)	18 (20.2)	0.001
	Male	48 (55.8)	71 (79.8)	
HT	Yes	52 (60.5)	69 (77.5)	0.023
	No	34 (39.5)	20 (22.5)	
Smoking	Yes	23 (26.7)	27 (30.3)	0.72
	No	63 (73.3)	62 (69.7)	
DM	Yes	30 (34.9)	35 (39.3)	0.652
	No	56 (65.1)	54 (60.7)	
Number of occluded vessels	1	-----	29 (32.6)	
	2	-----	30 (33.7)	
	3	-----	30 (33.7)	

CAD: Coronary Artery Disease; DM: Diabetes Mellitus; HT: Hypertension; n: Number of Participants; P: Significance Level Of Inter-Group

Table 2 FBS and lipid profile values belonging to control and CAD group

	Control	CAD	p
FBS	101.00 [93.27-124.85]	106.20 [91.46-136.97]	0.63
Total Cholesterol†	185.70 [160.00-217.20]	182.06 [140.00-219.80]	0.396
HDL-Cholesterol†	42.45±13.18	42.35±13.29	0.965
LDL-Cholesterol†	104.00 [91.00-139.00]	100.01 [74.32-129.58]	0.099
VLDL-Cholesterol†	33.00 [24.00-43.15]	32.02 [21.03-49.55]	0.887
Triglycerides*	128.60 [94.70-192.75]	153.10 [109.82-220.80]	0.121

† Continuous variables are presented as mean±standard deviation and concentration units are mg/dl.

*The parameters of the coefficients of variation that greater than 20% was evaluated by median values.

CAD: Coronary Artery Disease; FBS: Fasting Blood Sugar; P: Significance Level of Inter-Group.

Table 3 Hardy-Weinberg equilibrium for eNOS T786C variants in control and CAD group

	Genotypes	Observed Value	Expected Value	Chi-Square	P
Control	TT	54	51.42	2.515	0.113
	TC	25	30.16		
	CC	7	4.42		
CAD	TT	38	35.87	0.95	0.329
	TC	37	41.26		
	CC	14	11.87		

CAD: Coronary Artery Disease; eNOS: Endothelial Nitric Oxide Synthase; P: Significance Level of Inter-Group.

Table 4 eNOS T786C genotype distribution and the risks they create in control and CAD group

Genotypes	Control n (%)	CAD n (%)	OR	CI (%95)	p
TT	54 (62.8)	38 (42.7)	-----		
TC	25 (29.1)	37 (41.6)	2.1	1.092-4.051	0.026
CC	7 (8.1)	14 (15.7)	2.842	1.048-7.708	0.04
TT	54 (62.8)	38 (42.7)	----		
TC+CC	32 (37.2)	51 (57.3)	2.265	1.235-4.153	0.008
Allele frequency					
T	133 (77.3)	113 (63.4)	-----		
C	39 (22.7)	65 (36.6)	1.964	1.227-3.137	0.005

CAD: Coronary Artery Disease; CI: Confidence Interval; eNOS: Endothelial Nitric Oxide Synthase; n: Number of Participants; OR: Odds Ratio; P: Significance Level of Inter-Group.

Table 5 The relationship between the number of obstructed vessels and eNOS T786C genotypes in the CAD group

	Genotypes			p
Number of obstructed vessel	TT (%)	TC (%)	CC (%)	
1	15 (39.5)	12 (32.4)	2 (14.3)	0.11
2	14 (36.8)	13 (35.2)	3 (21.4)	
3	9 (23.7)	12 (32.4)	9 (64.3)	

CAD: Coronary Artery Disease; eNOS: Endothelial Nitric Oxide Synthase; p: Significance Level of Inter-Group.

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Table 6 Hardy-Weinberg equilibrium for eNOS G894T variants in control and CAD group

	Genotypes	Observed value	Expected value	Chi-square	p
Control	TT	8	6.42	0.735	0.391
	GT	31	34.16		
	GG	47	45.42		
CAD	TT	10	7.02	2.441	0.118
	GT	30	35.96		
	GG	49	46.02		

CAD: Coronary artery disease, eNOS: endothelial Nitric oxide synthase, p: Significance level of inter-group.

Table 7 eNOS G894T genotype distribution and the risks they create in control and CAD group

Genotypes	Control n (%)	CAD n (%)	OR	CI (%95)	p
GG	49 (55.1)	47 (54.7)	-----		
GT	30 (33.7)	31 (36.0)	1.077	0.566-2.047	0.82
TT	10 (11.2)	8 (9.3)	0.834	0.303-2.295	0.725
GG	49 (55)	47 (54.7)	----		
GT+TT	40 (45)	39 (45.3)	1.016	0.560-1.844	0.957
Allele frequency					
G	128 (71.9)	125 (72.7)	-----		
T	50 (28.1)	47 (27.3)	0.962	0.602-1.537	0.873

CAD: Coronary artery disease, eNOS: endothelial Nitric oxide synthase, CI: Confidence Interval, n: Number of participants, OR: Odds Ratio, p: Significance level of inter-group.

Table 8 The relationship between the number of obstructed vessels and eNOS G894T genotypes in the CAD group

	Genotypes			p
Number of obstructed vessel	TT (%)	GT (%)	GG (%)	
1	3 (30.0)	9 (30.0)	17 (34.7)	0.539
2	2 (20.0)	9 (30.0)	19 (38.8)	
3	5 (50.0)	12 (40.0)	13 (26.5)	

CAD: Coronary artery disease, eNOS: endothelial Nitric oxide synthase, p: Significance level of inter-group.

In this study, the data on risk factors derived from history of control and CAD groups were evaluated, and in parallel to the done previous studies[10] the mean age of the CAD group was determined to be higher than the control group (p=0.018). Similarly, male gender ratio that the studies recognized as risk factors alone, was found as 71% in patients with CAD, and 48% in control group (Table 1, p=0.001).

Most of risk factors that are related to atherosclerosis and cardiovascular morbidity and mortality, including traditional and nontraditional risk factors, were also found to be associated with endothelial dysfunction. Many of these risk factors, including hyperlipidemia, hypertension, diabetes, and smoking, are associated with overproduction of reactive oxygen species or increased oxidative stress.[11] In this study, there was no significant difference in the mean FBS, TC, HDL, LDL, VLDL and TG levels of the patients and control groups (Table 2). We believe that the cause of lack of statistically significant differences in these parameters was drug usage.

In the present study, in T786C polymorphism of eNOS, TT, TC and CC genotype frequencies were 42.7%, 41.6% and 15%, respectively, in CAD group and 62.8%, 29.1% and 8.1%, respectively, in the control group. Compared with the TT genotype, it was determined that those with TC genotype has 2100-fold (p= 0.026) and those with the CC genotype has 2.842 times (p=0.040) greater risk of developing coronary artery disease. Nakayama et al. demonstrated a point mutation at nucleotide −786 bp (T-786-C), in the 5'-flanking region of the eNOS gene.[12] They were suggested that this mutation, which results in a significant reduction in the promoter activity of eNOS gene, has been strongly associated with coronary spasm.[12]

The association of eNOS T786C polymorphisms with CAD was reported in many studies. In Italy, T-786C polymorphism of the eNOS gene was associated with the presence and severity of angiographically defined CAD.[13] Correspondingly, Kim et al.,[14] showed that T-786C polymorphism of the eNOS gene was associated with CAD and myocardial infarction.[14] Salimi et al.,[15] found that genotype frequencies of T-786C polymorphism in promoter were differed significantly between CAD patients and control group, and the frequency of the C allele of the T-786C polymorphism was significantly higher in CAD patients than in controls.[15] The large meta-analysis involving a total sample size of 69,235 subjects confirmed the association of the three NOS3 gene polymorphisms (Glu298Asp, −786 T/C, and 4 a/b) with the presence of CAD, The Glu298Asp polymorphism showed strongest association, followed by T786-C and 4b/a, in pooled analysis. Subgroup analysis revealed that Glu298Asp and 4b/a have highest degree of association amongst the Middle Easterners. Also, in this study T786-C and its minor allele seem to carry a highest risk for CAD among subjects of Asian ancestry.[16] In contrast, T-786C polymorphism of the eNOS gene was not associated with CAD in a study reported by Jaramillo et al.,[17] and Kincl et al.,[18]

In this study, in G894T polymorphism of eNOS, GG, GT and TT genotype frequencies were 55%, 36%, and 9%, respectively, in CAD group and 55%, 33% and 11%, respectively, in the control group. eNOS G894T polymorphism was shown no significant difference between CAD group and control group. Kamna et al.,[19] demonstrated the distribution of GG, GT and TT genotypes were found to be 71.22%, 28.06% and 0.72%, respectively, and the allelic frequency of G and T alleles were 0.853 and 0.148, respectively.[19] Syed et al. reported

that the distribution of Glu298/Asp (Glu/Glu, Glu/Asp and Asp/Asp) were 46.83%, 30.37% and 22.78%, respectively, in CAD subjects and 60.75%, 31.64% and 7.59%, respectively, in control. These results were significant between the controls and cases ($p < 0.05$).[20] Idrissi et al.,[21] found that eNOS G894T was significantly correlated to MI increased risk among Moroccan population.[21] Ben Ali M et al.,[22] reported that G894T eNOS polymorphism was associated with CAD under the dominant and additive models in Tunusian.[22]

Conclusion

CAD is a complex disorder that genetic and environmental factors have an important role in its development. Therefore, in addition to established risk factors, genetic risk factors may have important roles in the pathogenesis of coronary atherosclerosis and acute myocardial infarction.[23] The eNOS gene is a candidate risk factor for cardiovascular disease, since reduced NO synthesis has been shown to play a role in the development of coronary atherosclerosis.[24] Therefore, it was planned to determine the role of eNOS G894T and T786C polymorphisms with coronary artery disease in this study. In conclusion, the eNOS G894T genotypes were not associated with CAD and the eNOS T786C variants could be evaluated as a risk factor for CAD.

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Conflicts of interest

Author declares there is no conflicts of interest.

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