The relationship between endothelial nitric oxide synthase gene polymorphism (T-786 C) and coronary artery disease in the Turkish population

Abstract

Previous studies revealed that there were various mutations on endothelial nitric oxide synthase (eNOS) gene and these mutations might be a risk factor for coronary artery disease (CAD), myocardial infarction (MI), and hypertension (HT). In this study, we aimed to investigate the relationship between eNOS gene polymorphism (T-786 C) and coronary artery disease in the Turkish population. Two hundred and eleven unrelated individuals (152 male, 59 female, mean age 59 years, range 27–85) whose angiographic examinations were performed in our hospital were enrolled into the study; 159 of these had angiographically determined coronary artery lesions (≥50% stenosis at least in one vessel). Fifty-two individuals were free of coronary artery disease on their coronary angiography. The Gensini scoring system was used to determine the severity of the CAD. The polymerase chain reaction (PCR) method was used for genotyping the individuals. To determine the independent risk factors for coronary artery disease, multivariate logistic regression analysis was used. The variant distribution of the T-786 C polymorphism was as follows. For all individuals: TT 94 (44.5%), TC 88 (41.7%), CC 29 (13.8%); in CAD patients: TT 63 (39.6%), TC 73 (45.9%), CC 23 (14.5%); and in normal individuals: TT 31 (59.6%), TC 15 (28.8%), CC 6 (11.5%). There was a statistically significant difference in the variant distribution between CAD and normal individuals (P < 0.05). On the other hand, when we compared the frequency of the at-least-one-C-allele carriers (CC+TC, dominant model) and TT homozygous, those with at least one C allele were more prevalent in CAD patients. The results were as follows. In coronary artery disease patients: CC+TC 96 (60.4%), TT 63 (39.6%); in normals: CC+TC 21 (40.4%), TT 31 (59.6%) (P < 0.01). When we compared the allele distribution (T vs C, additive model) between CAD patients and normal controls, the results were as follows: T 0.625 vs 0.740, C 0.375 vs 0.260; there was also a statistically significant association between CAD and C allele (P < 0.05). When we compared the means of the Gensini scores between each genotype of the T-786 C mutation, there was a statistically significant difference. The results were TT (48.6 ± 37.3, median 43.0), TC (55.4 ± 41.2, median 41.0), CC (77 ± 43.6, median 80.0) (P < 0.05). Multivariate logistic regression analysis revealed that C-dominant (CC+TC) individuals had 2.9-fold more likelihood to suffer from CAD (odds ratio: 2.902; confidence interval: 1.272–6.622) (P < 0.05). We conclude that the T-786 C polymorphism of eNOS gene might be a risk factor for coronary artery disease in the Turkish population.

Key words

Nitric oxide · Coronary artery disease · Endothelial nitric oxide synthase gene · T-786 C polymorphism

Introduction

Nitric oxide (NO) is a vasoactive substance and a major mediator of endothelium-dependent vasodilatation, which is synthesized from l-arginine by endothelial nitric oxide synthase (eNOS) enzyme in the vascular endothelium. Nitric oxide diffuses from the endothelium to the vascular smooth muscle cells, where it increases the concentration of cyclic guanosine monophosphate (cGMP) by stimulating soluble guanyl cyclase, leading to vascular relaxation. Several studies suggest that the basal release of NO by the endothelium contributes to basal vascular tone. It has a key role in the regulation of vascular tone and blood pressure. In addition to its vascular smooth muscle relaxation effect, it inhibits adhesion of platelets and leukocytes to the
endothelium, reduces vascular smooth muscle cell migration and proliferation, and limits the oxidation of atherogenic low-density lipoproteins. Animal studies showed that chronic inhibition of eNOS enzyme accelerates atherosclerosis and results in elevated blood pressure. Moreover, deficient NO activity in the involved spasm arteries of patients with vasospastic angina has been reported.

Recently, investigators demonstrated several mutations in the eNOS gene and found that these polymorphisms differed largely among races due to large differences in the linkage pattern of −786T/C, Glu298Asp, and 4a/4b polymorphisms among races. It is believed that these mutations might result in altered NO metabolism and impaired NO release, leading to increased vascular tone and elevation in blood pressure. One of the mutations in the eNOS gene is a result of a thymidine (T) being replaced by a cytosine (C) at nucleotide −786 (T-786C). In this study, we aimed to investigate the relationship between T-786 C mutation of eNOS gene and CAD in the Turkish population.

**Materials and methods**

**Study population**

We studied 211 unrelated individuals (152 men, 59 women), mean age 59 years, range 27–85, consecutively who underwent coronary angiography due to acute coronary syndrome or whose cardiac ischemia was determined by noninvasive cardiac examination (treadmill test, myocardial perfusion synthiography). One hundred and fifty-nine of them had angiographically determined coronary artery lesions (≥50% stenosis at least in one coronary vessel). Fifty-two individuals were free of coronary artery disease on their coronary angiography as a control group. The control subjects who underwent coronary angiography due to valve replacement or atypical chest pain after noninvasive cardiac examination revealed the probability of cardiac ischemia, but their coronary angiography did not show critical stenosis (≥50% stenosis at least in one coronary artery). The Gensini scoring system was used to determine the extent and the severity of the CAD. All coronary artery segments involved by atheroma and the degree of their luminal narrowing (stenosis score 0%–25%, 25%–50%, 50%–75%, 75%–90%, 90%–99%, 100%) were determined and given a significance score of 1 to 2, respectively (1, 2, 4, 8, 16, 32) according to the geographic importance of the each vascular segment involved. After the significance scores are determined by assessing the percent narrowing of each coronary artery segment, they are multiplied by a factor according to regional importance of the coronary artery involved by atheroma, the left main coronary artery (LMCA) ×5, the proximal segment of the left anterior descending coronary artery (LAD) ×2.5, the mid-segment of the LAD ×1.5, the distal segment of the LAD ×1, the first diagonal branch of the LAD ×1, the second diagonal branch of the LAD ×0.5, the proximal segment of circumflex artery (Cx) ×2.5, the distal segment of the LCx ×1, obtuse marginal branch of the LCx ×1, the proximal segment of the right coronary artery (RCA) ×1, the mid-segment of the RCA ×1, the distal segment of the RCA ×1, the posterior coronary artery (PDA) ×1, the posterolateral artery ×1. Finally, the values of each coronary artery segment were added and the Gensini score was determined for all patients [Gensini score = sum of (stenosis score × functional significance score)]. The subjects were defined as hypertensive if their blood pressure was greater than 140/90 mmHg or if they were receiving any antihypertensive medication. Diabetes mellitus was defined if the individuals had a previous diagnosis, a history of antidiabetic use, or fasting glucose levels above 126 mg/dl. The subjects were considered to be hypercholesterolemic if their low-density lipoprotein (LDL) levels were above 130 mg/dl. Hypertriglyceridemia was considered if the triglyceride levels were above 150 mg/dl or if the participants were receiving any antilipidemic medications. Smoking status was classified as smokers or those who never smoked. Informed consent was obtained from all participants and the study protocol was approved by the ethics committee of our hospital.

**Genetic analysis**

Genomic DNA was extracted from peripheral lymphocytes by using phenol chloroform methodology. Screening for the NOS (−2786 T-C) substitution was performed by polymerase chain reaction (PCR)-RFLP (restriction fragment length polymorphism) methodology. The PCR reaction was carried out in a total 50-µl volume including 0.1–1 µg template DNA, 10× PCR buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, 10 pmol each primer (ENOS 5′-TGG AGA GTG CTG GTG TAC CCC A-3′ [forward] and ENOS 5′-GCC TCC ACC CCC ACC CTG TC-3′ [reverse]) and 1 U Taq polymerase (MBI Fermentas, Vilnius, Lithuania). Thirty-five cycles were performed as 2 min at 94°C for predenaturation, 1 min at 94°C for denaturation, 1 min at 62°C for annealing, 1 min at 72°C for extension, and 7 min at 72°C for the final extension. After PCR, the products obtained were digested with 10 U MspI restriction enzyme (MBI Fermentas) at 37°C overnight. After digestion, the fragments were run on 3% agarose gel electrophoresis at 90 V for 45 min. Subsequently the fragments of 40 bp (constant) and 140 (−786T) or 90 + 50 bp (−786C) were visualized with ethidium bromide under UV light.

**Statistical analysis**

SPSS 11.5 for Windows was used to perform all statistical analysis. The data are expressed as mean±SD. The comparison of the means of two continuous variables was made using an independent t-test. Cases who did not have a normal distribution were evaluated using Kruskal–Wallis analysis and the Mann–Whitney U-test. Chi-square analysis was used to compare the categorical variables. The means of Gensini scores of each genotype of the T-786 C mutation were compared using Kruskal–Wallis analysis. Multivariate logistic regression analysis was used to assess