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# Glu 298-Asp And T786-C Polymorphisms Of Endothelial Nitric Oxide Synthase Gene In Coronary Artery Disease Patients

## Koroner Arter Hastalığında Endotelyal Nitrik Oksit Sentaz Geninin Glu 298-Asp Ve T786-C Polimorfizmlerinin Araştırılması

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## ABSTRACT

**Aim:** We aimed to investigate the association of eNOS gene with Glu298-Asp and T-786C polymorphisms with Coronary artery disease (CAD) and to contribute to the elucidation of the genetic factors involved in the development of CAD.

**Patients and Methods:** A total of 200 patients were evaluated. Severe CAD was defined as ≥ 50% stenosis in at least one of major coronary arteries and these patients were taken into the CAD group (n=144). Patients without stenosis were included in the control group (n=66).

**Results:** After the evaluation of T-786C polymorphism, there was no significant difference between TT (p=0,660), TC (p=0,73) and CC (p=0,634) genotypes between CAD and control groups. There was no significant difference between the groups in both dominant (p=0,439) and recessive (p=0,622) model comparisons. When Glu 298-Asp polymorphism was examined, there was no statistically significant difference between GG (p=0,836), GT (p=0,581) and TT (p=0,767) genotypes when the groups were compared according to genotype distributions. The groups were statistically similar according to both dominant (p=0,697) and recessive (p=0,485) model com-parisons.

**Conclusion:** There was no statistical correlation between T-786 C and Glu 298-Asp polymorphisms and CAD. Similar studies with larger study populations should be conducted to clarify the role of T-786 C and Glu 298-Asp polymorphisms.

Key words: Endothelial nitric oxide synthase, coronary artery disease, nitric oxide, T-786C polymorphism, Glu298-Asp polymorphism

## ÖZ

**Amaç:** Bu çalışma ile eNOS geninin Glu298-Asp ve T-786C polimorfizmleri ile Koroner arter hastalığı (KAH) arasındaki ilişkisinin araştırılması ve KAH gelişmesinde rol oynayan genetik faktörlerin aydınlatılmasına katkıda bulunulması amaçlandı.

**Hastalar ve Yöntemler:** Çalışmada toplam 200 hasta değerlendirildi. Ciddi KAH, majör koroner arterlerin en az birinde ≥%50 darlık olması olarak tanımlanmış ve bu hastalar KAH grubuna alındı (n=144). Stenozisi olmayan hastalar kontrol grubuna dahil edildi (n=66).

**Bulgular:** T-786C polimorfizminin değerlendirilmesi sonucunda, KAH ve kontrol grupları karşılaştırıldığında TT (p=0,660), TC (p=0,73) ve CC (p=0,634) genotipleri arasında anlamlı fark saptanmamıştır. Her iki grupta da dominant (p=0,439) ve resesif (p=0,622) model karşılaştırmasında anlamlı fark yoktur. Glu 298-Asp polimorfizmi incelendiğinde; gruplar genotip dağılımlarına göre karşılaştırıldığında, GG (p=0,836), GT (p=0,581) ve TT (p=0,767) genotipleri arasında istatistiksel olarak anlamlı fark bulunmamıştır. Gruplar, hem dominant (p=0,697) hem de resesif (p=0,485) model karşılaştırmalarına göre istatistiksel açıdan benzer nulunmuştur.

**Sonuç:** Çalışma sonucunda, T-786 C ve Glu 298-Asp polimorfizmleri ve KAH arasında istatistiksel bir ilişki bulunmamıştır. T-786 C ve Glu 298-Asp polimorfizmlerinin rolünü açıklığa kavuşturmak için daha büyük çalışma popülasyonları ile benzer çalışmalar yapılmalıdır.

Anahtar Kelimeler: Endotelyal nitrik oksit sentaz, koroner arter hastalığı, nitrik oksit, T-786C polimorfizmi, Glu298-Asp polimorfizmi

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Coronary artery disease (CAD) is the leading cause of mortality and morbidity worldwide [1]. Coronary artery disease is caused by atherosclerotic and nonatherosclerotic causes, characterized by myocardial ischemia. Some risk factor of CAD can be modified by life-style changes like smoking, obesity and physical inactivity while lipid disorders, hypertension, diabetes mellitus and insulin resistance can be modified by both lifestyle changes and/or drugs. There are non-modifiable risk factors like age, gender and family history. Also lipoprotein a, homocysteine, c-reactive protein and fibrinogen are considered as new risk factors which plays important roles at beginning and progression of atherosclerosis [2-5]

Three different nitric oxide synthase (NOS) isoforms have been identified in mammalian cells: neuronal nitric oxide synthase (nNOS), inducible nitric oxide synthase (iNOS), and endothelial nitric oxide synthase (eNOS). Nitric oxide (NO) is the common product of NOS isoenzymes [6]. The main source of NO in circulation is the eNOS isoenzyme. The heart sends blood to blood vessels in each systole causing a mechanical effect on the surface of the endothelium of the blood vessels. This shear stress causes the synthesis of NO. As a result, NO is continuously produced from the endothelial cell [7, 8]. Small quantities of NO increases guanylate cyclase activity and physiological effects occur relative to the type of target cell. It is known that there is a continuous NO synthesis in the arterial system which provides a certain vessel tonus. The first event in endothelial dysfunction is the deterioration of endothelium-dependent vasodilatation via NO. Disorder in NO activity or production is suggested to be the main mechanism of endothelial dysfunction which triggers atherosclerosis [5, 9]. All of the factors that causes atherosclerotic plaque such as hypertension, dyslipidemia, smoking and diabetes mellitus are associated with abnormal endothelial functions and decrease in bioactive NO levels [10].

Variations have been identified in the intron, exon and promoter regions of the eNOS gene. T-786 C; is the point mutation caused by the exchange of the thymine (T) base at position 786 with the cytosine (C) base in the promoter region. Exon polymorphism of Glu298-Asp (G894T) occurs in exon 7 of the eNOS gene, resulting in displacement of the guanine (G) base at position 894 with thymine (T) base. In this situation, instead of the glutamate (Glu) amino acid normally expressed in position 298 of the eNOS gene, the aspartate (Asp) amino acid is replaced. Studies have been carried out suggesting that Glu 298-Asp or T-786 C polymorphisms are associated with atherosclerosis, causing diseases such as cerebrovascular diseases, CAD, myocardial infarction (MI) and many other diseases like Alzheimer's disease, metabolic syndrome, migraine and hypertension [11-16].

In this study, we evaluated patients admitted to coronary angiography with initial diagnosis of CAD. We aimed to investigate the association of eNOS gene with Glu298-Asp (Rs1799983) and T-786C (Rs2070744) polymorphisms with CAD and to contribute to the elucidation of the genetic factors involved in the development of CAD.

## PATIENTS AND METHODS

### Study Population

Patients with initial diagnosis of CAD and acute coronary syndrome (ACS) patients who referred to invasive laboratory were enrolled for this study. A total of 200 volunteers were included and patients with a history of malignity were excluded from the study.

Demographic parameters and clinical data were obtained from patient interviews and hospital medical records. Blood samples were taken from the peripheral vein after 8-12 hours of fasting period.

Total cholesterol higher than 200 mg/dl, LDL-C higher than 130 mg/dl, triglyceride higher than 150 mg/dL or patients under hyperlipidemia treatment were classified as hyperlipidemic patients. Patients with fasting blood sugar higher than 125 mg/dl or under anti-diabetic treatment are classified as diabetes mellitus patients. Hypertension diagnosis was defined as systolic blood pressures ≥140 mmHg, diastolic blood pressure ≥90 mmHg in at least two separate measurements or any antihypertensive drug use history. Smoking status was defined as; currently smoking or quitting smoking within a period of less than 1 year. Family story was defined as history of CAD before 55 years of age in first-degree male relatives and <65 years in first-degree female relatives.

Severe CAD was defined as ≥50% stenosis in at least one of major coronary arteries. According to CAD severity, patients were classified; one-vessel disease was ≥50% stenosis in left anterior descending artery (LAD), circumflex artery (Cx) or right coronary artery (RCA); two-vessel disease was ≥50% stenosis in two of LAD, Cx or RCA and three-vessel disease was ≥50% stenosis in LAD, Cx and RCA or isolated ≥ 50% stenosis in LMCA. Patients with two and three-vessel disease were classified as multivessel disease. Patients with stenosis in at least one of the major coronary arteries were grouped in CAD group. The patients with no stenosis were included in the control group.

Genetic Analysis

Blood samples for DNA isolation were obtained from the sheaths placed for coronary angiography and put in sterile tubes containing EDTA. The samples were stored at +4 °C. DNA was isolated from blood samples using DNA purification kit (Invitrogen, Germany).

The polymerase chain reaction (PCR) mixture for Glu298Asp (Rs17999983) was prepared in 200 µl PCR vials using 1.5 µl 3 mM MgCl, 1.5 µl 1x PCR buffer, 0.6 µl 0.2mM dNTP, 0.3 µl 1 nM T174M primer F (5'- AAG GCA GGA GAC AGTGGA TGG A-3'), 0.3 µl 1 nM T174M primer R (5'- CCC AGT CAA TCC CTTTGG TGC TCA-3'), 0.15 µl 1.25 units of Taq DNA Polymerase 17.65 µl and 3 µl isolated DNA. A total of 35 cycles of PCR program was performed consisting of denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min, and extension at 72 °C for 1 min for a cycle. 1 µl 10x Fast Digest GreenBuffer 0.5 µl 1x Fast Digestrestriction enzyme (MspI for G≥T (Glu298Asp)) 8 µl, 5 µl (~0.2 µg) PCR reaction product were used for G≥T (Glu298Asp) Restriction Fragment Length Polymorphism (RFLP) (ThermoScientific, Lithuania). The mixture was allowed to incubate at 37 °C for 1 hour. At the end of the incubation period, the products were run on a 2% agarose gel prepared with EtBr and the bands formed under UV light were observed and the genotype was decided.

Allelic polymorphisms can be explained as, Glu/Glu (GG); restriction region not found in both alleles (normal), Asp/Asp (TT); presence of the restriction site in both alleles (mutant) and Glu/Asp (GT); restriction site only in one allele (heterozygote).

The PCR mixture for T-786C (Rs2070744) was prepared in 200 µl PCR vials using 1.5 µl 3 mM $MgCl_2$ ,1.5 µl 1x PCR buffer, 0.6 µl 0.2mM dNTP, 0.3 µl 1 nM Primer F (5'- CACCTGCATTCTGGGA-ACTGTA 3 '), 0.3 µl 1 nM Primer R (5 '- GCCGCA-GTAGCAGAGAGACAC-3'), 0.15 µl 1.25 units of Taq DNA Polymerase 17.65 µl and 3 µl of isolated DNA. A total of 35 cycles of PCR program was performed consisting of full DNA denaturation at 94 °C for 1 min, denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min, and extension at 72 °C for 1 min for a cycle. The products were run on a 2% agarose gel prepared with EtBr and the bands formed under UV light were observed and the genotype was decided. In products with PCR end result; for the homozygous normal CC genotype, a single band of 176 bp; for the homozygous mutant TT genotype, 250 bp of product were generated. Two PCR products of 176 and 250 bp were gene-rated for the TC heterozygote genotype.
Ethical approval: Ethical approval was obtained from the local ethics committee.

Statistic Analysis: Statistical analysis was conducted using the SPSS version 20.0. Continuous variables, were expressed as arithmetic mean ± standard deviation and categorical variables were expressed as a percentage. Kolmogorov-Smirov and Shapiro-Wilk test were used as normality test. For binary comparison of continuous variables, Student t-test was used for those with normal distribution; non-parametric values were evaluated by Mann-Whitney U. Chi-square test was used to compare categorical variables. ANOVA model was used for comparisons across more than 2 groups with normal distribution and Kruskal-Wallis test was used for comparison of the groups with no normal distribution. Allele frequencies were calculated and assessed for Hardy-Weinberg equilibrium. The allele genetic model, the dominant model and the recessive model were established by calculating Odds ratio and 95% confidence intervals. Multivariate logistic regression analysis was used to determine independent risk factors for CAD. $P<0.05$ was considered statistically significant.

## RESULTS

A total of 200 cases were included in the study.

For the control group, 39 female and 27 male volunteers were included and 90 male and 44 female volunteers were included in the CAD group. A total of 100 patients had critical vascular disease. 50% (n=50) of these patients had one-vessel disease, 27% (n=27) had two-vessel diseases, and 23% (n=23) had three-vessel diseases.

The mean age of the control group was 52.9 ± 9.8 years and the mean age of the CAD group was 56.7 ± 8.2 years. Demographic and laboratory data of the control and CAD group were shown in Table 1.

According to the Hardy-Weinberg principle, alleles, genotypes and frequencies of generations remain constant in the gene pool of the population if there are no factors affecting the gene frequency. In the Hardy-Weinberg equilibrium analysis for the polymorphisms assessed in our study, the H1 hypothesis (population not balanced) was accepted because p values were lower than 0.05. The distributions of T-786 C and Glu 298-Asp alleles in control and CAD groups were observed to be incompatible with the Hardy-Weinberg equilibrium (Table 2).

eNOS gene T-786 C polymorphism

In the control group CC genotype was observed in 30.3% (n = 20) of subjects, TC genotype in 37.9% (p=25) of subjects and TT genotype in 31.8% (n=21) of subjects. In the CAD group, CC genotype was found in 35.8% (p=48) of patients, TC genotype in 32.1% (p=43) of patients and TT genotype 32.1% (p= 43) of patients. When the groups were compared according to genotype distributions, there was no statistically significant difference between TT (p=0.660), TC (p=0.73) and CC (p=0.634) genotypes. There was no significant difference between those with CC+TC genotype and those with TT genotype in dominant model comparison (p=0.439). There was no significant difference between those with TT+TC genotype and those with CC genotype in the recessive model comparison (p=0.622). When examined in terms of allele frequencies, 49.2% (p=65) T allele, 50.8% (p=67) C allele were detected in the control group and 51.9% (p=139) T allele and 48.1% (p=129) C allele were detected in the CAD group. There was no statistical significance between groups in terms of allele frequency (p=0.622). The relationship analysis between our groups for the T 786-C polymorphism was shown in Table 3.

No statistical correlation was found between T-786 C polymorphism and traditional atherosclerotic risk factors when we examined lipid profiles from demographic data and traditional atherosclerotic risk factors in the T-786 C polymorphism dominant model (Table 4).

Distribution of T-786 C polymorphism genotypes according to critical vessel disease incidences is shown in Table 5. There was no statistical significance in analysis between genotype distributions, dominant model and recessive model and critical vascular involvement (p=0.137, p=0.205, p=0.313, respectively)

eNOS gene Glu 298-Asp polymorphism

In the control group, GG genotype was observed in 48.5% (p=32), GT genotype in 15.1% (p=10) and TT genotype in 36.4% (p=24) of the subjects. In CAD group, GG genotype was observed in 53.7% (p=72), GT genotype in 12.7% (p=17) patients and TT genotype in 33.6% (p=45) patients. There was no significant difference between GG (p=0.836), TC (p=0.581) and CC (p=0.767) genotypes when the groups were compared according to genotype distributions. There was no significant difference between those who had GG+GT genotype and those who had TT genotype in comparison of dominant model (p=0.697). In the recessive model comparison, there was no statistically significant difference between those with TT+GT genotype and those with GG genotype (p=0.485). When examined in terms of allele frequencies, 56.1% (p=74) G allele, 43.9% (p=58) T allele were detected in the control group; In the CAD group, 60.1% (p=161) G allele and 39.9% (p=107) T allele were detected. There was no statistically significant difference in allele frequency between the groups (p=0.443) (Table 6).

The evaluation of the demographic data, laboratory data and the Glu 298-Asp polymorphism of the dominant model showed no statistical relationship between Glu 298-Asp polymorphism and traditional atherosclerotic risk factors (Table 7).

There was no statistical significance in analysis between genotype distributions, dominant model

**Table 1. Demographic and laboratory data of the control and coronary artery disease group**

| | Control Group (n=66) | CAD Group (n=134) | p |
|---|---|---|---|
| Age, years | 52.9±9.8 | 56.7±8.2 | 0.015 |
| BMI, kg/m2 | 27.6±4.5 | 28.5±4.4 | 0.186 |
| Waist circumference, cm | 97.9±12 | 100.2±13.7 | 0.215 |
| Total cholesterol, mg/dl | 185.2±37.1 | 191.2±40.6 | 0.317 |
| HDL-C, mg/dl | 52.8±13.1 | 42.1±10.9 | <0.001 |
| LDL-C, mg/dl | 117.5±33.5 | 124.7±40.6 | 0.181 |
| Triglyceride, mg/dl | 137.3±85.9 | 181±113.9 | <0.001 |
| Male, % | 27 (40.9) | 90 (67.2) | <0.001 |
| DM, % | 15 (22.7) | 44 (32.8) | 0.187 |
| HT, % | 29 (43.9) | 70 (52.2) | 0.294 |
| Smoking, % | 19 (28.8) | 57 (42.5) | 0.065 |
| Family History of CAD, % | 9 (13.6) | 37 (37.6) | 0.032 |

Abbreviations: BMI, body-mass index; CAD, coronary artery disease; DM, diabetes mellitus; HDL-C, high-density lipoprotein cholesterol; HT, hypertension; LDL-C, low-density lipoprotein cholesterol.

**Table 2. Hardy-Weinberg equilibrium in study groups**

| | Genotypes | Observed value | Expected value | Chi square | p |
|---|---|---|---|---|---|
| T-786 C | | | | | |
| Control Group | CC | 20 | 16 | 3.8 | 0.049 |
| | TC | 25 | 33 | | |
| | TT | 21 | 17 | | |
| | CC | 48 | 36 | | |
| CAD Group | TC | 43 | 67 | 17.1 | <0.001 |
| | TT | 43 | 31 | | |
| Glu 298-Asp | | | | | |
| Control Group | GG | 32 | 20.7 | 31.6 | <0.001 |
| | GT | 10 | 32.5 | | |
| | TT | 24 | 12.7 | | |
| | GG | 72 | 48.4 | | |
| CAD group | GT | 17 | 64.3 | 72.4 | <0.001 |
| | TT | 45 | 21.4 | | |

Abbreviations: CAD, coronary artery disease;

**Table 3. T-786 C genotype polymorphism relationship in control and coronary artery disease groups**

| T-786 C Genotype | Control Group n=66 | CAD | Relative Risk (Odd's Ratio) | Confidence Interval (%95) | p |
|---|---|---|---|---|---|
| CC | 20 (30.3%) | 48(35.8%) | 0.84 | 0.41-1.72 | 0.634 |
| TC | 25(37.9%) | 43(32.1%) | 1.17 | 0.56-2.4 | 0.673 |
| TT | 21(31.8%) | 43(32.1%) | 1(reference) | | 0.660 |
| Dominant Model | | | | | |
| CC+TC | 45(68.2%) | 91(67.9%) | 0.98 | 0.52-1.85 | 0.969 |
| TT | 21(31.8%) | 43(32.1%) | 1(reference) | | |
| Recessive Model | | | | | |
| TC+TT | 46(69.7%) | 86(64.2%) | 1.28 | 0.68-2.41 | 0.439 |
| CC | 20(30.3%) | 48(35.8%) | 1(reference) | | |
| Allele frequency | | | | | |
| C | 65(49.2%) | | 1.11 | 0.73-1.68 | 0.622 |
| T | 67(50.8%) | | 1(reference) | | |
| | | | | | |
| | | | | | |

Abbreviations: CAD, coronary artery disease;

**Table 4. Comparison of demographic and laboratory data according to T-786C dominant model**

| | CC+TC n=136 | TT n=64 | p |
|---|---|---|---|
| Age, years | 55.1 ± 8.3 | 56.2 ± 10 | 0.375 |
| BMI, kg/m2 | 28.2 ± 4.3 | 28.1 ± 4.8 | 0.804 |
| Waist circumference, cm | 99.9 ± 13.7 | 98.5 ± 12.2 | 0.496 |
| Total cholesterol, mg/dl | 188.5 ± 42.7 | 190.8 ± 43.5 | 0.716 |
| HDL-C, mg/dl | 45.6 ± 12.8 | 45.7 ± 12.3 | 0.536 |
| LDL-C, mg/dl | 121 ± 38.4 | 125.1 ± 38.8 | 0.481 |
| Triglyceride, mg/dl | 169.4 ± 113 | 160.7 ± 94.5 | 0.861 |
| Male, % | 76 (55.9) | 41 (64) | 0,273 |
| DM, % | 39 (29) | 20 (31.3) | 0.710 |
| HT, % | 34 (32) | 35 (55) | 0.314 |
| Smoking, % | 51 (37.5) | 25 (29.1) | 0.832 |
| Family History of CAD, % | 33 (24.3) | 13 (21.4) | 0.536 |

Abbreviations: BMI, body-mass index; CAD, coronary artery disease; DM, diabetes mellitus; HDL-C, high-density lipoprotein cholesterol; HT, hypertension; LDL-C, low-density lipoprotein cholesterol

Table 5. Distribution of T-786C polymorphism genotypes according to critical vascular involvement

| T-786 C | One-vessel disease n=50 | Multivessel disease n=50 | p |
|---|---|---|---|
| CC | 20 (40%) | 13 (26%) | 0.137 |
| TC | 16 (32%) | 17 (34%) | |
| TT | 14 (28%) | 20 (40%) | |
| Dominant Model | | | |
| CC+TC | 36 (36%) | 30 (30%) | 0.205 |
| TT | 14 (14%) | 20 (20%) | |
| Recessive Model | | | |
| TT+TC | 24 (24%) | 19 (19%) | 0.313 |
| CC | 26 (26%) | 31 (31%) | |
| | | | |

and recessive model and critical vascular involvement (p=0.435, p=0.391, p=0.313, respectively) (Table 8).

We included 59 people with ACS in our study population. The genotype distribution of these persons is given in Table 9.

When we examined the relationship between ACS and polymorphisms, there was no statistically significant relationship between T-786 C polymorphism (p=0.620) and Glu 298-Asp polymorphism (p=0.120) and ACS (Table 10).

## DISCUSSION

In this study, we investigated the relationship between Glu 298-Asp and T-786 C polymorphisms of the eNOS gene and CAD. We aimed to investigate the disease-genetic relationship. In conclusion, there was no relationship between CAD and both T-786 C and Glu 298-Asp polymorphisms of eNOS gene.

Recently, a number of studies have been conducted to investigate whether the Glu 298-Asp or T-786 C polymorphisms of the eNOS gene are associated with CAD. In some studies, it has been shown that Glu 298-Asp polymorphism is a risk factor for the presence and prevalence of CAD [17, 18]. Also some studies suggest that there was no relationship between Glu 298-Asp polymorphism and CAD [19]. Data on whether eNOS polymorphisms are risk factors for CAD differ in studies conducted in different populations. In studies conducted in France, Ireland, Canada, India and Chile, there was no correlation between Glu 298-Asp polymorphism and CAD [20-23]. Casas et al. [24] conducted a meta-analysis evaluating 26 studies and found that 298-Asp polymorphism and intron 4a/4b mutations were associated with increased risk for CAD. In our study, there was no relationship between CAD and Glu 298-Asp polymorphisms. Also the incidence of Asp/Asp genotype in the CAD group was found to be 33.6% which is higher than other studies. In the study group, mutant (24% Asp/Asp) and heterozygote (10% Glu/Asp) genotypes were found in a relatively high proportion compared to other populations. Even the frequency of Glu 298-Asp mutant gene and allele was higher, it is not related to CAD. Whether or not there is a defect in NO production in all patients with cardiac symptoms is still a matter of debate.

Tangurek et al.[18] investigated the relationship between T-786 C polymorphisms of eNOS gene and CAD and reported that T-786 C mutation could be evaluated as a risk factor for the presence and prevalence of CAD. Casas et al. [24] reported that there was no relationship between T-786C polymorphism and CAD. In our study, there was no statistical correlation between CAD and eNOS T-786 C polymorphisms. Fatini et al. [25] studied Glu 298-Asp, intron 4a4b and T-786C polymorphisms of the eNOS gene in 477 patients with ACS and a control group of 537 patients and found relationships between 4a4b and T-786C polymorphisms for ACS but Glu 298-Asp polymorphism did not show a significant relationship. In patients with ACS, the incidence of Glu/Glu genotype was 38.8%, the Glu/Asp genotype frequency was 44.6% and the Asp/Asp genotype frequency was 16.5%. In our study, there was no correlation between Glu 298-Asp and T-786 C polymorphism and ACS. These results were partially parallel to the results of Fatini et al. In our study,

**Table 6. Glu 298-Asp genotype polymorphism relationship in control and coronary artery disease groups**

| Glu 298-AspGenotype | Control Groupn=66 | CAD Group n=134 | Relative Risk (Odd's Ratio) | Confidence Interval (%95) | p |
|---|---|---|---|---|---|
| GG | 32 (48.5%) | 72 (53.7%) | 0.90 | 0.36-2.28 | 0.836 |
| GT | 10 (15.1%) | 17 (12.7%) | 1.2 | 0.62-2.29 | 0.581 |
| TT | 24 (36.4%) | 45 (33.6%) | 1(reference) | ----- | 0.767 |
| Dominant Model | | | | | |
| GG+GT | 42 (63.6%) | 89 (66.4%) | 0.88 | 0.47-1.63 | 0.697 |
| TT | 24 (36.4%) | 45 (33.6%) | 1(reference) | | |
| Recessive Model | | | | | |
| TT+GT | 34 (51.5%) | 62 (46.3%) | 0.81 | 0.44-1.46 | 0.485 |
| GG | 32(48.5%) | 72 (53.7%) | 1(reference) | | |
| Allele frequency | | | | | |
| G | 74 (56.1%) | 161 (60.1%) | 1.17 | 0.77-1.79 | 0.443 |
| T | 58 (43.9%) | 107 (39.9%) | 1(reference) | | |

**Table 7. Comparison of demographic data according to Glu 298 Asp dominant model**

| | GG+GT, n=131 | TT, n=69 | p |
|---|---|---|---|
| Age, years | 55.7±8.7 | 55±9.3 | 0.852 |
| BMI, kg/m2 | 28.2±4.6 | 28.2±4.3 | 0.999 |
| Waist circumference, cm | 99.6±12.9 | 99.1±13.9 | 0.813 |
| Total cholesterol, mg/dl | 190.7±42.4 | 186.4±43.8 | 0.502 |
| HDL-C, mg/dl | 46±13.2 | 45±11.5 | 0.605 |
| LDL-C, mg/dl | 122.3±40.2 | 122.4±35.2 | 0.991 |
| Triglyceride, mg/dl | 165.9±115.1 | 167.9±91.5 | 0.396 |
| Male, % | 78(%60) | 39(%56.6) | 0.680 |
| DM, % | 42(%32.1) | 17(%24.7) | 0.274 |
| HT, % | 66(%50.4) | 33(%47.9) | 0.731 |
| Smoking, % | 53(%40.5) | 23(%33.4) | 0.324 |
| Family History of CAD, % | 31(%23.7) | 15(%21.8) | 0.758 |

Abbreviations: BMI, body-mass index; CAD, coronary artery disease; DM, diabetes mellitus; HDL-C, high-density lipoprotein cholesterol; HT, hypertension; LDL-C, low-density lipoprotein cholesterol

**Table 8. Distribution of Glu 298-Asp polymorphism genotypes according to critical vascular involvement**

| Glu 298-Asp | One-vessel disease n=50 | Multivessel disease n=50 | p |
|---|---|---|---|
| GG | 28 (56%) | 29 (58%) | 0.435 |
| GT | 5 (10%) | 6 (12%) | |
| TT | 17 (34%) | 15 (30%) | |
| Dominant Model | | | |
| GG+GT | 32 (32%) | 36 (36%) | 0.391 |
| TT | 18 (18%) | 14 (24%) | |
| Recessive Model | | | |
| TT+GT | 24 (24%) | 19 (19%) | 0.313 |
| GG | 26 (26%) | 31 (31%) | |

Abbreviations: CAD, coronary artery disease;

**Table 4. Comparison of demographic and laboratory data according to T-786C dominant model**

| | CC | | TC | | TT | |
|---|---|---|---|---|---|---|
| | Control Group n=20 | CAD Group n=48 | Control | CAD Group n=43 | Control Group n=21 | CAD Group n=43 |
| ACS | 1(%1.7) | 18(%30.5) | 2(%3.4) | | 1(%1.7) | 16(%27.1) |
| | GG | | GT | | TT | |
| Genotype distribution of Glu 298-Asp according to acute coronary syndrome | | | | | | |
| | Control Group n=32 | CAD Group n=72 | Control Group n=10 | CAD Group n=17 | Control Group n=24 | CAD Group n=45 |
| ACS | 3(%5.1) | 33(%55.9) | 0 | 4(%6.8) | 1(%1.7) | 18(%30.5) |

Abbreviations: ACS, acute coronary syndrome; CAD, coronary artery disease.

**Table 10. Comparison of genotypes of polymorphism with acute coronary syndrome**

| T-786 C | | | | |
|---|---|---|---|---|
| | CC n=68 | TC n=68 | TT n=64 | p=0.620 |
| ACS positive | 19 | 23 | 17 | |
| Glu 298-Asp | | | | |
| | GG n=104 | GT n=27 | TT n=69 | p=0.120 |
| ACS positive | 36 | 4 | 19 | |

Abbreviations: ACS, acute coronary syndrome

Glu/Glu (GG) genotype frequency was found to be 61%, Glu/Asp (GT) genotype frequency was 6.8% and Asp/Asp (TT) genotype frequency was 32.2% in patients with ACS. We found that the frequencies of GT and TT genotypes at lower percentages than the normal genotype GG so the Glu298-Asp variation was considered to have no relationship with CAD.

According to GENICA study [26], there was a statistically significant relationship with the T-786 C polymorphism and CAD patients with one, two and three-vessel disease. In our study, there were no statistical correlations when patients with critical vascular disease were classified as one and multivessel disease in the CAD group. The same study assessed the C allele with multivariate logistic regression together with other coronary risk factors, and found that patients with C allele had 3.6 times more risk of CAD. In our study, the CC genotype did not make a statistical difference even though allele frequency were high in the CAD group. There was no statistical difference, although there was still a large number of at least one C allele (CC+TC) in the CAD group. We evaluated the patients with epicardial critical vascular lesions in our statistical analysis. Carriers with at least one C allele (CC+TC) were evaluated according to one and multivessel disease and C allele frequency found to be higher in one-vessel patients and no statistically significant correlation was found. The lack of statistical association of vascular involvement with polymorphisms suggests that there is no link between the severity of CAD and these mutations.

According to Hardy-Weinberg analysis, it was assumed that the population was not balanced because the p values were 0.05 smaller. In our groups, it was observed that distributions of T-786 C and Glu 298-Asp alleles did not fit the Hardy-Weinberg distribution. The reason why Hardy-Weinberg analysis showed no statistical difference in our study population may be explained by the random selection of the spouses of the individuals and also migration to the community.

Major limitation of the study was small study population. Also this study is conducted in one health center so this study population only represents one region of the country.

As a conclusion: In this study, we investigated the relationship between Glu 298-Asp and T-786 C polymorphisms of the eNOS gene and CAD. There was no correlation between CAD, number of epicardial artery involvement, acute coronary syndrome and both eNOS T-786 C and eNOS Glu 298-Asp polymorphisms. Similar studies with larger study populations and more diverse ethnic groups should be conducted to clarify the role of T-786 C and Glu 298-Asp polymorphisms.

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