

# The NOS3 T-786C (rs2070744) Gene Polymorphism in Patients of Uzbek Nationality with Chronic Heart Failure

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## Abstract

This study aimed to evaluate the role of endothelial nitric oxide synthase (eNOS) T-786C (rs2070744) gene polymorphism in chronic heart failure (CHF) manifestation in Uzbek patients. The study involved 81 CHF male patients, Uzbeks, aged from 41 to 70 years. The control group comprised 75 healthy, age-matched, randomly selected male persons. Genomic DNA was isolated and SNP genotyping was performed by using a polymerase chain reaction with specific primers followed by restriction fragment length polymorphism analysis. In CHF patients, the observed frequency of genotypes was as follows: TT=0.556; CT=0.432; CC=0.012; the expected frequency of genotypes was as follows: TT=0.595; CT=0.352; CC=0.052 ( $\chi^2=4.14$ ,  $P=0.04$ ). Deviation from Hardy–Weinberg equilibrium was noted due to an excess of heterozygosity. The results of our study have shown a significant association between CT genotype of NOS-3 T-786C gene polymorphism and CHF manifestation in patients of Uzbek nationality.

**Keywords:** eNOS; T-786C (rs2070744) gene polymorphism; chronic heart failure.

## Introduction

Studies of the human genome have made possible early diagnosis not only of genetic diseases but also of many multifactorial diseases. In practice, this goal can be achieved by molecular testing of candidate genes. In CHF development, activation of the sympathetic-adrenal system (SAS) and the renin-angiotensin-aldosterone system (RAAS) plays a key role [1,2]. In the study of genes involved in the formation of CHF, the research of gene polymorphism of the SAS and RAAS components has a special interest. Along with this, endothelial dysfunction also plays a central role in the development and progression of a number of cardiovascular diseases and HF [3-6]. Endothelial function and nitric oxide availability affect myocardial function, systemic and pulmonary hemodynamics,

and coronary and renal circulation. Arterial stiffness modulates ventricular loading conditions and diastolic function, key components of heart failure with preserved ejection [7].

NO is a primary physiological transmitter derived from the endothelium, and plays a composite role with diverse anti-atherogenic effects as vasodilator [8]

In normal vascular physiology, NO plays a key role to maintain the vascular wall in a quiescent state by inhibition of inflammation, cellular proliferation, and thrombosis.

Reduced bioavailability of nitric oxide (NO) and abundant formation of reactive oxygen species (ROS) within the vascular wall are the key determinants in endothelial dysfunction. The imbalance between NO and ROS mainly results from neurohumoral activation associated with heart failure. As endothelial derived NO is a major endogenous modulator of platelet function, reduced intravascular bioactivity of NO contributes to platelet activation, adhesion and thromboembolic events in heart failure [9-12].

NO is synthesized from L-arginine by means of endothelial nitric oxide synthase (eNOS, type 3), an isoform of NOS, which is predominant in the walls of the blood

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vessels. The eNOS gene localized to 7q 35-36, comprises of 26 exons spanning 26 kilo bases and encodes an mRNA of 4052 nucleotides. The NOS3 gene harbors many polymorphic sites including SNPs, variable number tandem repeat (VNTR) sequences etc. The most examined and functionally related polymorphisms are T-786C of the 5'UTR region, G894T in exon7 and 27 bp VNTR polymorphism in intron 4 respectively [13-16]. Variable expression of NOS3 enzyme due to NOS3 gene polymorphisms have been reported to be a significant contributor to cardiovascular morbidity and mortality [17]. The T-786C functional polymorphism in the promoter region is linked with decreased eNOS expression. This polymorphism has been reported to be associated with retinopathy in type 1 diabetes [18]] and cardiovascular diseases such as hypertension and coronary arterial spasm [19,20].

This study aimed to evaluate the role of NOS3 T-786C (rs2070744) gene polymorphism in CHF manifestation in Uzbek patients.

## Material and Methods

The study involved 81 CHF (ischemic genesis) male patients, Uzbeks, aged from 41 to 70 years. The control group comprised 75 healthy, age-matched, randomly selected male persons. All the patients were divided into three groups according to the New York Heart Classification (NYHA) functional class (FC). NYHA FC was determined by the 6-minute walk test (6MWT). All patients underwent clinical examination, ECG, and echocardiography. Group 1 consisted of 12 patients with CHF FC-I, Group 2 consisted of 30 patients with CHF FC-II, and Group 3 consisted of 39 patients with CHF FC-III.

Genomic DNA was extracted from peripheral blood using the Diatom™ DNA Prep 200 Kit according to the manufacturer's protocol. Polymerase chain reaction restriction fragment length polymorphism-based (PCR-RFLP) techniques and visualization were employed and performed to determine the NOS3 T-786C (rs2070744) polymorphism. PCR amplification was carried using thermocycler CG-1-96 «Corbett Research» (Australia) and «Medlab» kits (St. Petersburg, Russia) according to the manufacturer's protocol.

The following sequence primers were used:

F: 5'- CTGTGGACCAGATGCCAG-3'

R: 5'- GTCATTACAGTGACGCACGCTC-3'

The PCR condition was as follows: denaturation at 95° C -5 min; 37 cycles: 95° C -30 sec, 60° C -30 sec, 72° C-1 min, and final synthesis - 5 min. The PCR product was digested with MspI enzyme (Merck, Germany). The genotypes were identified as TT (230/25 bp), CT (230/184/46/25 bp), and CC (184/46/25 bp) respectively.

Statistical analysis was performed using a statistical software package, «GenePop». Chi square ( $\chi^2$ ) or Fischer's exact test (two sided) was used to compare the association between the genotypes and alleles in relation to the cases, and test for deviation of genotype distribution from Hardy-Weinberg equilibrium. The odds ratio (OR) and their 95 % confidence intervals (CI) were calculated to estimate the

strength of the association. A value of  $P < 0.05$  were considered statistically significant.

## Results

Genotype and allele frequencies of NOS3 T-786C polymorphism in CHF patients and control group are shown in Table 1.

**Table 1.**

**The distribution of allele and genotype frequencies of NOS3 T-786C gene polymorphism in CHF patients and control group**

| Groups           | Alleles        |               | Genotypes     |               |              | HWE<br>$\chi^2$ , df=1<br>(P) |
|------------------|----------------|---------------|---------------|---------------|--------------|-------------------------------|
|                  | T              | C             | T/T<br>% (n)  | T/C<br>% (n)  | C/C<br>(n)   |                               |
| CHF<br>FC I      | 20<br>(0.833)  | 4<br>(0.167)  | 8<br>(0.667)  | 4<br>(0.333)  | -            | 0.48<br>(0.49)                |
| CHF<br>FC II     | 44<br>(0.733)  | 16<br>(0.267) | 15<br>(0.500) | 14<br>(0.467) | 1<br>(0.033) | 1.12<br>(0.29)                |
| CHF<br>FC III    | 61<br>(0.782)  | 17<br>(0.218) | 22<br>(0.564) | 17<br>(0.436) | -            | 3.03<br>(0.08)                |
| Total            | 125<br>(0.772) | 37<br>(0.228) | 45<br>(0.556) | 35<br>(0.432) | 1<br>(0.012) | 4.14<br>(0.04)                |
| Control<br>group | 128<br>(0.853) | 22<br>(0.147) | 53<br>(0.707) | 22<br>(0.293) | -            | 2.22<br>(0.14)                |

In the control group, the observed frequency of genotypes was as follows: TT=0.707; CT=0.293; CC=0; the expected frequency of genotypes was as follows: TT=0.728; CT=0.250; CC=0.022 ( $\chi^2=2.22$ ,  $P=0.14$ ). The observed frequency of genotypes was in Hardy-Weinberg equilibrium.

In CHF patients, the observed frequency of genotypes was as follows: TT=0.556; CT=0.432; CC=0.012; the expected frequency of genotypes was as follows: TT=0.595; CT=0.352; CC=0.052 ( $\chi^2=4.14$ ,  $P=0.04$ ). Deviation from Hardy-Weinberg equilibrium was noted due to an excess of heterozygosity. The frequencies of T and C alleles were 0.772:0.228 in patients versus 0.853:0.147 in the control group ( $P > 0.05$ ). Comparative analysis showed a significant association between the CT genotype and CHF manifestation in Uzbek patients according to an additive model of inheritance ( $\chi^2= 4.20$ ,  $P=0.04$ ; OR=1.83; 95% CI: 0.94-3.56).

In patients with CHF FC-I, the observed frequency of genotypes was as follows: TT=0.667; CT=0.333; CC=0; the expected frequency of genotypes was as follows: TT=0.694; CT=0.278; CC=0.028 ( $\chi^2=0.48$ ,  $P=0.49$ ). The frequencies of T and C alleles were 0.833:0.167 in patients versus 0.853:0.147 in the control group ( $\chi^2=0.07$ ,  $P=0.8$ ; OR=1.16; 95% CI: 0.36-3.73).

In patients with CHF FC-II, the observed frequency of genotypes was as follows: TT=0.500; CT=0.467; CC=0.033; the expected frequency of genotypes was as follows: TT=0.538; CT=0.391; CC=0.071 ( $\chi^2=1.12$ ,  $P=0.29$ ). The frequencies of T and C alleles were 0.733:0.267 in patients versus 0.853:0.147 in the control group ( $\chi^2= 4.16$ ,  $P=0.04$ ;

OR=2.12; 95% CI: 1.02-4.39). Comparative analysis showed a significant association between the T/C genotype and CHF manifestation in Uzbek patients according to additive model of inheritance ( $\chi^2=4.94$ ,  $P=0.03$ ; OR=2.11; 95% CI:0.88-5.05).

In patients with CHF FC-III, the observed frequency of genotypes was as follows: TT=0.564; CT=0.436; CC=0; the expected frequency of genotypes was as follows: TT=0.612; CT=0.341; CC=0.048 ( $\chi^2=3.03$ ,  $P=0.08$ ). The frequencies of T and C alleles were 0.782:0.218 in patients versus 0.853:0.147 in the control group ( $\chi^2=1.84$ ,  $P=0.18$ ; OR=1.62; 95% CI: 0.80-3.27).

The differences between the observed and expected frequencies of heterozygosity ( $D=(\text{hobs}-\text{hexp})/\text{hexp}$ ) for control group and group of patients are shown in Table 2. The observed heterozygosity significantly prevailed than the expected one in CHF patients.

**Table 2.**

**The differences between the observed and expected frequencies of heterozygosity for NOS-3 T-786C gene polymorphism in CHF patients and control group**

| Groups     | The observed heterozygosity | The expected heterozygosity | D     |
|------------|-----------------------------|-----------------------------|-------|
| CHF        | 0.432                       | 0.352                       | 0.23* |
| CHF FC I   | 0.333                       | 0.278                       | 0.20  |
| CHF FC II  | 0.467                       | 0.391                       | 0.19  |
| CHF FC III | 0.436                       | 0.341                       | 0.28  |
| Control    | 0.293                       | 0.250                       | 0.17  |

\*-  $P<0.04$

**In conclusion**, the results of our study have shown a significant association between CT genotype of NOS3 T-786C gene polymorphism and CHF manifestation in patients of Uzbek nationality. Our findings suggest that the presence of the NOS3 gene mutant allele reduces endothelial production of NO in vessels and predisposes the post-MI patients carrying the mutant C allele to CHF manifestation with a decrease in LV contractility and development of systolic dysfunction.

## Competing interests

The authors declare that they have no competing interests.

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