Acta Cardiol Sin 2017;33:420–428
Original Article
doi: 10.6515/ACS20161215B

General Cardiology

The Endothelial Nitric Oxide Synthase (NOS3–786T>C) Genetic Polymorphism in Chronic Heart Failure: Effects of Mutant -786C allele on Long-term Mortality

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Background: Nitric oxide plays an important role in the regulation of basal vascular tone and cardiac myocyte function. We investigated the NOS3–786T>C polymorphism in chronic heart failure (CHF) and its effects on long-term mortality.

Methods: Ninety-one patients with CHF who were referred to the Department of Cardiology of Siyami Ersek Cardiovascular and Thoracic Surgery Center for cardiopulmonary exercise testing between April 2001 and January 2004 and 30 controls were enrolled in this study. Patient were followed prospectively for a period of 1 to 12 years.

Results: Patients and controls were divided into three groups: TT, TC and CC, according to their NOS3–786T>C polymorphism. We noted that there was no significant difference in the genotype distribution between patients and controls. There was also no significant difference in endothelial nitric oxide synthase (eNOS) gene polymorphism between ischemic HF and nonischemic HF. During the follow-up period, 61 (67%) deaths occurred. The nonsurvivor group had lower left ventricular ejection fraction (LVEF) ($p = 0.01$), reduced peak oxygen consumption ($p = 0.04$) and were of older age ($p = 0.001$). Age, LVEF, peak oxygen consumption and genotype were found to be predictors of mortality ($p < 0.05$). Additionally, mortality was significantly increased in -786CC genotype patients compared to TT genotype patients (hazard ratio = 2.2; $p = 0.03$). By multivariate analysis, age and eNOS genotype were determined to be significant independent predictors of death. Additionally, Kaplan-Meier analysis confirmed that homozygote -786C genotype was associated with an increased risk of death ($\chi 2 = 4.6$, $p = 0.03$).

Conclusions: Our findings showed that the NOS3–786T>C polymorphism was associated with an increased risk of mortality in patients with CHF.

Key Words: Chronic heart failure • Endothelial nitric oxide synthase • Long-term mortality • NOS3–786T>C gene polymorphism

INTRODUCTION

Nitric oxide (NO) is formed directly from the amino acid of L-arginine by the catalytic reaction of different isoforms of NO synthases (NOS). Three distinct isoforms of the NOS enzyme, derived from separate genes, neural NOS (nNOS), inducible NOS (iNOS) and endothelial (eNOS), have been identified in humans.[1] Both eNOS and nNOS are constitutively expressed enzymes and produce small amounts of NO on stimulation. The eNOS is expressed in endothelial cells, endocardial cells and cardiomyocytes, whereas the nNOS isoform is expressed in neural cells and skeletal muscle.[2,3] iNOS is another

Received: June 17, 2016 Accepted: December 15, 2016
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isoform that is expressed in many cell types, mainly in response to inflammatory cytokines. Low to moderate levels of the NO produced by iNOS prevents free radical-related damage, but high levels of NO not only may be toxic to undesired microbes, parasites or tumor cells but also may harm healthy cells.[4] The reversible myocardial depression seen in septic shock and other inflammatory states have been attributed to NO production by inducible myocardial NOS.

Previous study has shown that NO attenuates cardiac myocyte contraction, inhibits β-adrenergic responses, enhances diastolic function and reduces O_2 consumption.[5] NO diminishes the effect of α-agonist stimulation on contractility, potentially limiting the impact of sympathetic activation on disease progression. Sympathetic activation serves as an important short-term compensatory mechanism to maintain stable hemodynamic in heart failure (HF), but persistent sympathetic activation precipitates worsening ventricular remodeling and HF.[6] Given the effects on adrenergic response and the peripheral vasculature, it may be postulated to have a cardioprotective effect in HF. Several polymorphisms have been identified in the eNOS gene, and have been associated with cardiovascular disorders. Among them, a polymorphism in the 5-flanking region of the eNOS gene (NOS3–786T>C) was found to be associated with increased risk for coronary spasms, coronary artery disease (CAD), and acute myocardial infarction (MI).[7-9] Mutant C allele implies a blunted transcription rate and is far more common in CAD.[7,8] In the present study, we investigated the prevalence of the NOS3–786T>C polymorphism of the eNOS gene in patients with chronic heart failure (CHF) and their impact on long-term survival.

SUBJECTS AND METHODS

Ninety-one consecutive patients [mean age 59 ± 12 years (range, 31 to 76), 82% male] with CHF [left ventricular ejection fraction (LVEF) < 40%] who were referred to the Department of Cardiology of Siyami Ersek Cardiovascular and Thoracic Surgery Center for cardiopulmonary exercise testing between April 2001 and January 2004 were prospectively enrolled in this study. We evaluated the role of the NOS3–786T>C polymorphism in predicting long-term mortality for this cohort. Inclusion criteria were clinical stability for at least 3 months and absence of signs of acute cardiac decompensation. Patients with valvular disease, chronic renal failure, cancer or other severe concomitant disease and a condition limiting exercise (joint pain, peripheral artery disease) were excluded from the study. All patients were on optimized medical therapy (Table 2). The study patients were followed through the outpatient HF clinic of our institution at 3 and 6 months. Informed consent was obtained from each patient, and the study was also approved by the institutional review board. Demographic information, peak oxygen consumption (peak VO_2), LVEF and medical therapy were recorded. Patients were followed prospectively for a mean period of 70 ± 43 months. Mortality data was obtained through outpatient clinical attendance records and telephone interviews with the patients or with the patients' primary care physician. Patients with angiographic evidence of CAD (defined as > 50% stenosis of a major epicardial coronary artery) were classified as ischemic. Left ventricular dysfunction was due to ischemic heart disease in 54 patients. The remaining 37 patients were identified as patients with nonischemic cardiomyopathy (NICM). The control group was comprised of 22 men and 8 women who had normal physical examination, electrocardiography, echocardiography, chest radiography and routine blood chemistry. The mean age of the control subjects was 57 ± 8 years (range, 38-71 years), which was not statistically different from the mean age of patients with CHF.

Echocardiographic examination

Transthoracic echocardiography was performed in all patients to measure LVEF at rest in the left lateral decupitus position by using ultrasound equipment (Vivid 7.GE Echopac sysytem GE, USA) with a 2.5 MHz transuser. The LVEF by two-dimentional echocardiography was derived from the apical four and two-chember views according to the modified biplane Simpson's method.[10]

Cardiopulmonary exercise testing (CPET)

In all patients, symptom-limited CPET was performed to assess maximal exercise capacity according to the ramp protocol by using a respiratory gas analysis system (Quinton 5000, Seattle, Washington USA) and Cortex 3B

apparatus (Nonnenstrasse, Leipzig, Germany) for measuring breath-by-breath O_2 uptake and CO_2 production.[11] Exercise testing was started at a constant speed of 1.7 m.n.h at 0^0 slope, with 2^0 further increments in slope per minute until the patient was limited by complaints of shortness of breath, fatigue, or chest discomfort. The system was calibrated with a standard gas mixture of known concentration before each test. Peak oxygen uptake (peak VO_2) was defined as the highest VO_2 observed during the exercise test and was expressed in ml/min/kg. Heart rate at baseline and at peak exercise were recorded. Resting heart rate was defined as the lowest heart rate recorded in the seated position before exercising, and peak heart rate the highest heart rate achieved during exercise.

Analysis of the NOS3–786T>C polymorphism in the 5′-flanking region of the eNOS gene

Genomic DNA was isolated from peripheral lymphocytes by using phenol chloroform methodology. Oligonucleotide primers 5′-TGG AGA GTG CTG GTG TAC CCC A-3′ (forward) and 5′-GCC TCC ACC CCC ACC CTG TC-3′ (reverse) were used for PCR. 35 PCR cycles were performed for 2 minutes at 94 °C for predenaturation, 1 min at 94 °C for denaturation, 1 minute at 62 °C for annealing, 1 minute at 72 °C for extension and 7 minute at 72 °C for final extension. After PCR, the amplified products were digested with MspI restriction enzyme (MBI Fermantas, Lithuania) at 37 °C overnight producing fragments of 140 and 40 bp for the wild type allele (allele T) or 90, 50 and 40 bp in the case of a polymorphic variant (allele C). After digestion, the fragments were run in 2% agarose gel electrophoresis and visualized with ethidium bromide under UV light.[12]

Statistical analysis

The results are expressed as the mean ± standard deviation (SD) for the continuous variables and as absolute numbers and precentages for categorical datas. To assess differences between the mean of the two continuous variables, the Student's t-test was performed. Differences between categorical variables, genotype distribution and Hardy-Weinberg (HW) equilibrium were tested by chi-square analysis. Univariate and multivariable Cox proportional hazards regression analysis was used to identify independent predictors of mortality. The initial selection of the variables entered in the model was based on univariate analysis significance with inclusion criteria of $p < 0.1$. The results of the Cox proportional hazards model are presented as the hazard ratio (HR). Kaplan-Meier cumulative mortality curves were also plotted, and comparison between groups of patients with the NOS3–786T>C genotypes variance were performed by log-rank test. The main analysis used to evaluate association of the HF and prognosis with the NOS3–786T>C genotypes variance was performed under a co-dominant genetic model represented by heterozygous comparison of TC vs. TT and homozygous comparison of CC vs TT. A two-tailed p value < 0.05 was considered as statistically significant. All analyses were performed using the Statistical Package for the Social Sciences, Version 22 (SPSS Inc, Chicago, IL, USA).

RESULT

The study group was divided into three groups: TT, TC, CC, according to their NOS3–786T>C

Polymorphisms in the 5-flanking region of the eNOS gene. Genotype absolute numbers and frequencies are shown in Table 1. The distributions of the NOS3–786T>C genotypes in the patients and the controls were found to be in HW equilibrium ($\chi 2 = 1.1$, $\chi 2 = 0.9$, respectively). In co-dominant model statistical analysis, there was no significant difference in the genotype distribution between patients and controls. In the control group, the frequencies of TT, CT and CC genotype were

Table 1. Genotype absolute number and frequency of the NOS3–786T>C polymorphism in patients and controls

	Genotype		
	TT	CT	CC
	no (%)		
Controls (n = 30)	20 (66)	8 (27)	2 (7)
CHF (n = 91)	49 (54)	32 (35)	10 (11)
NICM (n = 37)	19 (52)	12 (32)	6 (16)
Ischemic HF (n = 54)	30 (56)	20 (37)	4 (7)

Values are n (%).
No significant association was found between the NOS3–786T>C polymorphism and NICM and ischemic HF ($p > 0.05$).
CHF, chronic heart failure; HF, heart failure; NICM, nonischemic cardiomyopathy; NOS, nitric oxide synthase.

66%, 27% and 7%, respectively, and in the HF group 54%, 35%, 11%, respectively (p > 0.5). Prevalence of the -786 TT homozygotes was slightly but not significantly higher in controls as compared with those of patients (p > 0.05). We also found no significant differences in eNOS gene polymorphism between ischemic HF and nonischemic HF. The clinical characteristics of the patients classified according to the genotype distribution were shown in Table 2. There were no significant differences with respect to male sex, hypertension (HT), diabetes mellitus (DM), exercise capacity, etiology of HF and use of medications. However, LVEF was lower in patients with CC genotype (p = 0.008).

Survival analysis: During follow-up, 61 (67%) deaths occurred, 6 being in the first year. One patient died from a noncardiac cause (malignancy). Of the 60 cardiac deaths, 22 had a sudden cardiac death, and 38 were due to progressive HF. Clinical data, echocardiographic, cardiopulmonary parameters and hemodynamic characteristics between the survivor and non-survivor groups were shown in Table 3. No significant differences were found between the survivor and non-survivor groups for sex, HT, DM, etiology of HF, NYHA class, heart rate and medications. However, those who died had lower LVEF (p = 0.01), reduced peak VO_2 (p = 0.04) and of older age (p = 0.001). Genotype frequencies of the investigated NOS3–786T>C gene polymorphism in the survivors and non-survivors are given in Table 4. The mortality rate was 61% in patients carrying genotype TT, 69% in patients carrying genotype TC and 90% in patients carrying genotype CC (p > 0.05). In the univariate cox regression analysis, age, LVEF and peak VO_2 were found to be pre-

Table 2. Clinical characteristics of chronic heart failure patients classified according to NOS3–786T>C genotype

	TT (n = 49)	TC (n = 32)	CC (n = 10)	p value
Age (years)	58 ± 13	60 ± 11	61 ± 12	0.7
Male (%)	80	90	70	0.4
Hypertension (%)	28	28	33	0.9
Diabetes mellitus (%)	14	12	11	0.9
CAD	61	62	40	0.4
Mean LVEF (%)	28 ± 6	25 ± 7	22 ± 5	0.008
Peak VO_2 (ml/min//kg)	14 ± 5	15 ± 4	14 ± 3	0.8
Rest HR (beats/min)	86 ± 13	86 ± 17	84 ± 11	0.9
Peak HR (beats/min)	137 ± 20	137 ± 23	136 ± 21	0.9
Medication				> 0.05
ACE inhibitor or ARB (%)	94	84	90	
Beta-blocker (%)	80	84	80	
Aldosterone antagonist (%)	43	40	50	
Digitalis (%)	33	15	30	
Diuretic (%)	43	28	40	

ACE, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blockers; CAD, coronary artery disease; HR, heart rate; LVEF, left ventricular ejection fraction; NOS, nitric oxide synthase; Peak VO_2, peak oxygen consumption.

Table 3. Comparison of the various variables between patients with survivors and nonsurvivors

Variable	Survivors (n = 30)	Nonsurvivors (n = 61)	p value
Age	53 ± 13	63 ± 10	0.001
Male/female	22/8	53/8	0.1
Hypertension	7	17	0.5
Diabetes mellitus	4	9	0.7
İschemic HF	11	26	0.6
Mean LVEF (%)	29 ± 6	25 ± 5	0.01
Peak VO_2 (ml/min//kg)	15.9 ± 3.7	13.8 ± 4.8	0.04
Rest HR (beats/min)	86 ± 10	86 ± 17	0.9
Peak HR (beats/min)	137 ± 22	137 ± 21	0.9
NYHA class			> 0.05
Class 1 (%)	46	40	
Class 11 (%)	31	33	
Class 1l1 (%)	23	27	
Medication			> 0.05
ACE inhibitor or ARB (%)	94	87	
Beta-blocker (%)	86	77	
Aldosterone antagonist (%)	46	44	
Digitalis (%)	26	27	
Diuretic (%)	40	36	

ACE, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blockers; HF, heart failure; HR, heart rate; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; Peak VO_2, peak oxygen consumption.

Table 4. Genotype distribution of the NOS3-786T>C polymorphism between the survivors and non-survivors in chronic heart failure patients

	Genotype (%)		
	TT (N = 49)	CT (N = 32)	CC (N = 10)
	no (%)		
Survivors (n = 30)	19 (39)	10 (31)	1 (10)
Non-survivors (n = 61)	30 (61)	22 (69)	9 (90)
		p = 0.204	

Values are no (%).
NOS, nitric oxide synthase.

dictors of mortality. There was also a significant association between of the NOS3–786T>C polymorphism of the eNOS gene and survival under a homozygous model (CC versus TT). Risk of death was significantly higher in CC genotype [HR = 2.2, 95% confidence interval (CI): 1.05-4.7, p = 0.03; Table 5]. TC carriers (heterozygous model, TC versus TT), also had an increased risk of death, but which was not statistically significant (HR: 1.4, 95% CI: 0.86-2.6, p = 0.15). When these variables were entered into a Cox proportional hazards regression model, only age (HR = 1.06, 95% CI: 1.02-1.09, p = 0.002) and CC genotype (HR = 2.4, 95% CI: 1.01-5.6, p = 0.04) were found to be independent predictors of mortality (Table 6). Kaplan-Meier event-free survival curve confirmed that the NOS3–786T>C polymorphism was associated with an increased risk of death. CC genotype was associated with a significantly worse long-term prognosis (CC vs. TT genotypes, $\chi 2$ = 4.6, p = 0.03). Patients with -786CC genotype had a lower survival rate compared with -786 TT patients (10% vs. 39%, Figure 1).

DISCUSSION

The present study is the first to define a role for the NOS3–786T>C polymorphism of the eNOS gene in predicting outcome of patients with CHF in Turkish population. The results of this study suggest that the NOS3–786T>C mutation confers an increased risk of death in patients with CHF. Mortality rate has been found to be significantly higher in-786 CC homozygote patients.

Previous studies have described that the NOS3–786T>C mutation in the 5′ flanking region of the eNOS gene suppresses eNOS gene transcription.[7,8,13,14] The decrease in eNOS transcription is consistent with the notion that endothelial NO production is reduced in patients carrying the -786 C allele.[7] Basal generation of NO by eNOS plays an important role in the regulation of basal vascular tone and blood pressure.[15,16] In addition, NO regulates leukocyte-endothelial cell interaction, inhibits platelet aggregation, limits smooth muscle cell migration and proliferation, limits the oxidation of atherogenic low-density lipoproteins and affects cardiac myocyte function.[17-19] Reduced or impaired synthesis of NO, due to genetic polymorphism in eNOS may therefore promote development of cardiovascular disease. Several studies have reported a positive association between the NOS3–786T>C polymorphism and CAD.[7,9,20,21]

However, limited data exist on the relationship between this variable and HF. The NOS3–786T>C variant's role in disease susceptibility has remained unclear, with the findings showing various and sometimes contradictory results. The results of our study demonstrated that there was no significant difference in the genotype frequencies between patients and controls. We also didn't find any significant difference in genotype distribution between ischemic and nonischemic HF patients. Similar

Table 5. Predictors of survival by univariate Cox regression analysis

Variable	Hazards ratio	95% CI	p value
Age	1.04	1.016-1.069	0.001
LVEF %	0.95	0.91-0.99	0.04
Peak VO_2 (ml/min//kg)	0.93	0.87-0.98	0.01
CC vs. TT genotype	2.2	1.05-4.7	0.03
TC vs. TT genotype	1.4	0.86-2.6	0.15

LVEF, left ventricular ejection fraction; Peak VO_2, peak oxygen consumption.

Table 6. Predictors of survival by multivariate Cox regression analysis

Variable	Hazards ratio	95% CI	p value
Age	1.06	1.02-1.09	0.002
LVEF	0.98	0.93-1.03	0.5
Peak VO_2 (ml/min//kg)	0.99	0.9-1.07	0.7
CC vs. TT genotype	2.4	1.01-5.6	0.04

LVEF, left ventricular ejection fraction; Peak VO_2, peak oxygen consumption.

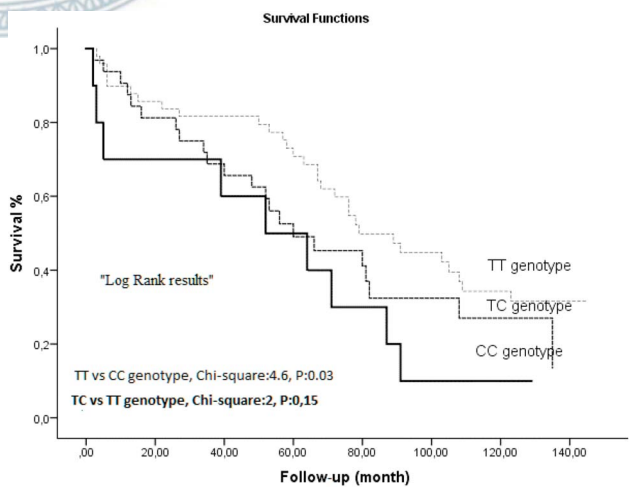

Figure 1. *Kaplan-Meier survival curve.*

to our results, Vecoli et al. have demonstrated that the genotype distribution of the NOS3– 786T>C polymorphism in patients with ischemic and NICM was not significantly different from that observed in the control group.[22] However, a significant association of TC genotype was observed in patients with dilated cardiomyopathy (DCM) in a study by Matsa et al. The risk of DCM was about 1.7-fold higher in subjects carrying the TC genotype of the NOS3–786T>C polymorphism.[23] On the other hand, Martinelli et al. found a marked ethnic difference in the distribution of eNOS variant. They reported that the genotype frequencies of the NOS3–786T>C polymorphisms in Caucasian HF patients were similar to those of controls. In the same study, haplotype frequencies composed of three eNOS polymorphisms (T-786C, VNTR4a/b and Glu298Asp) were different between the HF and control patients, among African-Brazilians ethnicity, with C allele being more frequent in controls than in patients with CHF.[24]

Furthermore, it has been reported that there was a significant association between genotype of the NOS3–786T>C gene polymorphism and CHF manifestation.[25] As endothelium derived NO is a major endogenous modulator of platelet function, reduced intravascular bioactivity of NO contributes to platelet activation, adhesion, and thromboembolic events in HF.[26,27] Moreover, anti-platelet activity of the drug has been shown to be decreased and cardiovascular events have been found to be increased in the presence of variant alleles.[20,28,29] The NOS3–786T>C polymorphism has also been shown as an independent determinant of insulin resistance in both ischemic and NICM patients.[22] In addition, post MI patients carrying the mutant C allele has shown predisposition to development of systolic dysfunction and HF in Uzbek nationality.[25] Patients with CHF who are homozygous for -786 C polymorphism of the eNOS gene have also shown advanced autonomic imbalance.[30] The prevalence of atrial fibrillation was high in CHF and the patients with gene polymorphism may have higher atrial fibrillation susceptibility than the others.[31,32] Thus, this polymorphism may serve as a marker for patients at increased risk for sudden death and more rapid progression of disease.[25-32] Our study revealed a strong association between the NOS3–786T>C polymorphism and outcome in Turkish patients with HF. Mortality was highest in patients who were homozygote for the C allele. TC carriers also had increased risk of death compared to normal homozygotes, but the risk was not significant. The mortality rate was observed to be 61% in TT786 homozygotes, 69% in TC carriers and 90% in -786C homozygotes.

Reported data with regard to prognostic association of HF with eNOS polymorphisms has been inconsistent. There is a marked ethnic difference in the effect of eNOS polymorphisms on survival. Martinelli et al. evaluated several polymorphisms in patients with HF, including NOS3–786T>C, Glu298Asp, intron 4, and has failed to demonstrate any association of these polymorphisms with prognosis in both Caucasian and African-Brazilian subjects. In addition, they failed to demonstrate any significant effect of eNOS polymorphisms on survival in the analysis restricted to Caucasian patients. On the contrary, African-Brazilian patients that carry the Glu298 variant had a worse survival rate when compared to Asp 298 carriers.[24] Similarly, Tardin et al. did not establish any association between the Glu298Asp polymorphism and prognosis in the sample of Brazilian patients with HF.[33] However, McNamara et al. have reported that the presence of the NOS3 asp298 variant was associated with poorer event-free survival in HF patients.[34] The Asp 298 variant increased the relative risk of event compared with Glu homozygotes (RR = 1.39; 95% CI, 1.03 to 1.86; p = 0.029).

These findings have strengthened the concept that eNOS variability in CHF may be related to the etiology of HF and racial phenotype. A meta-analysis has revealed that glu298asp and 4b/a have the highest degree of association amongst Middle Eastern countries.[35] Among subjects of Asian ancestry, the NOS3–786T>C polymorphism has been found to carry the highest risk of cardiovascular disease.[35] Heymes et al. have shown an association between the left ventricular gene expression of eNOS and iNOS and the extent of left ventricular dysfunction in patients with dilated cardiomyopathy.[5] This finding has been supported by a study in a pacing-induced chronic HF dog model, in which after an early increase in eNOS activity, cardiac NO production was reduced after 4 weeks of pacing.[36] These investigations have demonstrated that an increase in NO production might play an important role in HF syndrome.

Loss of NO bioavailability is a cardinal feature of endothelial dysfunction, and HF is associated with an en-

dothelial dysfunction of coronary arteries as well as large conductance and peripheral arteries.[26,27] The -786C allele has been associated with a significant reduction in eNSO3 gene transcription.[7,13,14] Thus, it has been suggested that the -786C variant might have an important role in the outcome of CHF patients.

Transient release of low doses of NO by stimulation of eNOS both from endothelial cells and myocytes may play an important role on the extent of left ventricular dysfunction in HF. Stimulation of cardiac NO production from the coronary endothelium has been shown to enhance left ventricular relaxation and reduce left ventricular stiffness.[5] Thus, cardiac production of NO may have a beneficial role in left ventricular dysfunction by maintaining the Frank-Starling mechanism.[37] Transient release in low dose of the NO by eNOS modulates load-dependent relaxation and autoregulation of myocardial perfusion. Patients with HF have diminished functional eNOS capacity compared with those without HF, both at rest and during submaximal exercise.[38] Scerrer-Crosbie et al. have found end-diastolic and end-systolic volumes to be increased, whereas fractional shortening, contractility, and survival are decreased in eNOS-deficient mice compared with wild-type after 4 weeks of left anterior descending artery ligation.[39] Jones et al. have demonstrated a protective role of NO derived from eNOS in infarct-induced congestive HF. Overexpression of eNOS exerts beneficial regional effects on pulmonary blood flow. Cellular release from eNOS inhibits myocardial oxygen consumption.[40] Endogenous NO spares myocardial oxygen consumption through attenuation of LV contractile response to β-adrenergic stimulation.[41] Enhanced NO production in HF may serve as an oxidant scavenger during HF, thereby minimizing the deteriorative effects of superoxide and other reactive oxygen species.[40] However, NO acts in a biphasic and in a dose-dependent manner on the regulation of apoptosis. Physiologically relevant levels of NO seem to suppress the apoptotic pathway. However, higher levels of NO may overwhelm the protective mechanism and exert proapoptotic and cytotoxic effects.[42]

Study limitations

Our study did have certain limitations. First, we analyzed the NOS3–786T>C polymorphism in patients with heterogeneous disease states, such as CAD, hypertension and diabetes. These might have affected the disease progression, gene distribution and outcome in our study group. Second, our data was a relatively small sized cohort of HF patients and controls. Moreover, we performed an analysis of specific pre-defined polymorphism, instead of genome-wide analysis. Lastly, our study group was derived from a cohort of CHF patients refered to a center for CPET study; thus, the results cannot be generalized to all patients with impaired left ventricular systolic funtion in the community.

CONCLUSIONS

We showed that the NOS3–786T>C gene variant was associated with poor long-term survival independent of the LVEF or peak VO_2 in Turkish patients with CHF. The presence of the homozygote -786CC genotype can be a useful genetic marker for identifying individuals at an increased risk of death. On the other hand, genotype of the NOS3–786T>C polymorphisms showed no significant differences between CHF patients and controls. Genotype frequencies of ischemic HF patients were also comparable with nonischemic HF patients. To summarize, we suggest that the NOS3–786T>C polymorphism did not play a role in the development of HF. However, -786CC genotype was associated with increased mortality rate in patients with CHF.

CONFLICT OF INTEREST

This project did not receive any financial support. The authors declare that they do not have any conflict of interest to disclose.

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