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Association of genetic polymorphisms with endothelial dysfunction in chronic heart failure

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Abstract. – OBJECTIVES: Endothelial dysfunction can be shown very early in the cardiovascular disease. In the present study the association between congestive heart failure (CHF), endothelial function and 3 gene polymorphisms was investigated.

PATIENTS AND METHODS: In 104 healthy controls and 104 CHF patients, endothelial constitutive nitric oxide synthase (ecNOS), angiotensin converting enzyme (ACE) and angiotensin II type 1 receptor (AT1R) gene polymorphisms were assessed. The cause of CHF was ischemic in 68 patients and dilated cardiomyopathy (DCMP) in 36 patients. High resolution brachial artery ultrasound was used in 37 CHF patients and 37 healthy controls to assess the endothelial function. Endothelium-dependent vasodilation (EDD) and endothelium-independent vasodilation (EID) were determined.

RESULTS: There no was difference between controls and CHF patients for the ACE, ecNOS, and AT1R genotype frequencies. Compared to controls CHF patients had significantly impaired EDD (9.0+5% vs 16±7%, $p < 0.001$) and EID (13±6% vs 19+8%, $p = 0.001$). EDD (7±4% vs 12+6%, $p = 0.005$), but not EID, was significantly impaired in ischemic CHF as compared to DCMP patients. In the CHF group ecNOS a allele and AT1R C allele influence the EDD.

CONCLUSIONS: Endothelial dysfunction was present in CHF group and the presence of ecNOS a allele and AT1R C allele further impaired EDD.

Key Words:
Endothelial function, Brachial artery ultrasound, Gene polymorphisms, Chronic heart failure.

Introduction

Endothelial dysfunction is now recognized as an early, perhaps initiating event in the pathogenesis of coronary artery disease (CAD), and has been shown to be present in patients with cardiovascular risk factors, and in chronic heart failure (CHF)[1-5]. Evaluation of endothelium-dependent (EDD) and endothelium-independent vasodilation (EID) has shown that endothelial function of the peripheral conduit and resistance vessels is impaired in CHF patients[6-7]. The origin of endothelial dysfunction in CHF lies in the interaction between genetic predisposition and environmental influences. Recent studies indicate that endothelial dysfunction is related to abnormalities in nitric oxide (NO) and in the activation of the renin-angiotensin system[8]. At present, very limited data are available regarding the allele frequencies of genes of these two systems, namely, endothelial constitutive NO synthase (ecNOS), angiotensin-converting enzyme (ACE) and angiotensin II type I receptor (AT1R) in patients with CHF. Particularly, their role in the endothelial dysfunction has been less extensively studied.

Therefore, the aim of the present study was to evaluate the possible relationships between the gene polymorphisms and the endothelium dysfunction in healthy volunteers and in CHF patients.

Patients and Methods

Study Population

A series of 104 patients (71 males, mean age 63±10) with heart failure caused by systolic dysfunction referred to the University Hospital of Istanbul (Departments of Internal Medicine and of Cardiology) were enrolled. As control group, 104 healthy subjects (61 males; mean age 45+11) were selected from the Outpatient Clinic. Informed consent was obtained from each subject and the Declaration of Helsinki on Biomedical Research on Humans was followed for the study. At the time of study entry demographic informa-

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tion, cardiovascular evaluation and medical therapy were recorded. In all patients the most recent clinical assessment demonstrated left ventricular systolic dysfunction, defined as left ventricular ejection fraction (LVEF) < 40 (mean LVEF 34±5%). In all patients LVEF was estimated by echocardiography. All patients enrolled had previously undergone diagnostic evaluation for CAD that consisted of coronary angiography (> 90%) and noninvasive assessment in the remainder. Patients with angiographic evidence of coronary disease (defined as stenosis > 50% in a major epicardial coronary artery) or a noninvasive assessment positive for ischemia or previous myocardial infarction were classified as ischemic. In our study population 68 patients were ischemic, whereas 36 showed dilated cardiomyopathy (DCMP). Table I resumes the clinical characteristics of study population.

Laboratory Analysis

Major risk factors for CAD according to the National Cholesterol Education Program[9] criteria were evaluated. Venous blood samples were drawn after 12 to 14 hours overnight fast for laboratory analysis, DNA isolation and genotyping. Serum total cholesterol, low density lipoprotein, triglycerides, high density lipoprotein, uric acid, fibrinogen, hemograms and electrolytes were measured by standard methods in the clinical laboratory department of the University Hospital of Istanbul and recorded. Obesity was defined as body mass index (BMI) > 27 kg/m^2.

Mutation Analysis and Nomenclature

DNA was extracted from peripheral blood lymphocytes by salting-out method. Three gene polymorphisms were investigated.

ecNOS4a/b Polymorphisms

The genotyping for NOS3 intron 4 a/b alleles was done by polymerase chain reaction methods as described previously, with some minor modifications[10]. In this polymorphism we observed 4 (a allele) or 5 times (b allele) the 27-basepair variable number of tandem repeats in intron 4.

ACE Gene I/D Polymorphism

The ACE gene I/D polymorphism was genotyped according to the methods described by Rigat et al[11]. To exclude mistyping of the heterozygotes as DD homozygotes all the DD genotypes samples were confirmed with an insertion specific PCR[12].

Table I. Clinical characteristics of study population.

Characteristics	Controls group (n = 104)	CHF group (n=104)	p1	DCMP group (n=36)	Ischemic CHF group (n=68)	p2
Age (years)	45 ± 11	63 ± 10	< 0.01	64 ± 13	63 ± 8	0.6
Male sex no. (%)	61 (58)	71 (68)	0.2	19 (53)	52 (76)	0.3
Current smokers no. (%)	41 (39)	55 (53)	0.07	11 (30)	44 (65)	0.1
Diabetes mellitus no. (%)	0 (0)	36 (35)	< 0.01	8 (22)	28 (41)	0.2
Family history of CAD no. (%)	26 (25)	46 (44)	< 0.01	15 (42)	31(45)	0.8
BMI > 27kg/m^2 no. (%)	37 (35)	31 (30)	0.5	11 (30)	20 (29)	0.9
Laboratory analysis						
TC > 200 mg/dl no. (%)	15 (14)	38 (36)	< 0.01	13 (36)	25 (37)	0.9
LDL > 130 mg/dl no. (%)	15 (14)	25 (24)	0.2	5 (14)	20 (29)	0.09
HDL < 35 mg/dl no. (%)	22 (21)	39 (37)	0.02	12 (33)	27 (40)	0.7
TG > 200 mg/dl no. (%)	12 (11)	25 (24)	0.03	4 (11)	21 (31)	0.04
UA > 7.5 mg/dl no. (%)	1 (1)	27 (26)	< 0.01	6 (17)	21 (31)	0.2
Fibrinogen > 400 mg/dl no. (%)	10 (10)	58 (56)	< 0.01	18 (50)	40 (59)	0.4
Medical therapy						
Aspirin no. (%)	0 (0)	97 (93)	< 0.01	30 (83)	67 (99)	0.6
ACE inhibitors no. (%)	0 (0)	91 (88)	< 0.01	30 (84)	61 (90)	0.8
ß-blockers no. (%)	0 (0)	35 (34)	< 0.01	6 (17)	29 (43)	0.06
Statins no. (%)	0 (0)	28 (27)	< 0.01	4 (11)	24 (35)	0.04
ATR blockers no. (%)	0 (0)	13 (12)	< 0.01	6 (17)	7 (10)	0.5
Diuretics no. (%)	0 (0)	81 (78)	< 0.01	32 (89)	49 (72)	0.5

CHF = congestive heart failure. DCMP = dilated cardiomyopathy. CAD = coronary artery disease. TC = Total cholesterol. LDL = Low density lipoprotein. HDL = High density lipoprotein. TG = Triglyceride. UA = Uric acid. ACE = angiotensin converting enzyme. ATR = angiotensin II receptor.

AT1R Gene A1166C Polymorphism

The genotyping for A1166C of AT1R gene was done as described previously[13]. The polymorphism consists to an A-C transversion at nucleotide position 1166 of the mRNA sequence, giving three different possible genotypes: AA, AC, or CC.

Assessment of Endothelial Function

Endothelial function was assessed in 74 subjects, 37 of CHF group and 37 of control group (age and sex matched). Their baseline characteristics did not differ from those of entire study population (data not shown). Brachial artery was imaged on a commercially available ultrasound system (Vingmed Technology, System Five, Horten, Norway) using a 10.0 MHz linear phased-array ultrasound transducer longitudinally just above the antecubital fossa. Blood pressure cuff was wrapped around the upper arm, inflated to 250 mmHg and held for 5 minutes to induce ischemia. The cuff was released and brachial artery diameter was measured every minute for 5 minutes to assess maximal EDD in response to reactive hyperemia. After vessel diameter returned to baseline values (~ 7-10 minutes), EID was assessed after 0.5 mg sublingual nitroglycerine, every minute for 5 minutes. Vessel diameters were measured at the end diastole coincident with the onset of the R-wave of the simultaneously obtained ECG trace. During the measurements particular attention was paid to the age, temperature of the laboratory, menstrual cycle, exercise, drugs, food, and sympathetic stimuli. The percent vasodilation was calculated with the following formula: Percent EDD or EID = 100* (pBAD- bBAD)/(bBAD), where pBAD was the peak brachial artery diameter after intervention and bBAD the baseline brachial artery diameter. The intra and inter-observer variability of the measurements in our laboratory was 1-3%. Our team for brachial artery ultrasound evaluation consisted of two experienced physicians (O.H., G.H.).

Statistical Analysis

Continuous data are presented as means ± SD, with the significance of differences judged by independent samples Student's *t* test. Categorical variables were summarized in terms of number and percentages and were compared using with % test. For 2 × 2 contingency tables Yates correction was done. If expected frequencies in the cells of 2'2 table were less than 5, Fisher's exact test was used. In the groups of subjects investigated the frequencies of genotypes were compared by chi-square analysis. Spearman's correlation coefficients were used to detect any association between variables. Factors that affect EDD and EID were examined with multiple regression tests. Probability was significant at a level of < 0.05. Analysis was performed using STATISTICA 6.1 (StatSoft Inc, Tulsa, OK, USA) and with a commercially available Statistical Package for Social Sciences for Windows ver 10.0 (SPSS Inc, Chicago, IL, USA).

Results

Cardiovascular risk factors of patients and controls are shown in Table I. The patients and controls were not similar with respect to distribution of age, diabetes mellitus and family history of CAD. Also cholesterol, uric acid and fibrinogen were higher in the CHF group than controls. Also between ischemic CHF and DCMP patients, we observed any differences in the clinical characteristics and laboratory analysis (Table I). Pharmacological treatment of patients is shown in Table I. All patients with CHF were under treatment for the duration of 53±42 months.

Polymorphisms Analysis

The genotype frequencies of ACE, AT1R, and ecNOS genes are given on Table II. The distribution of the genotypes was in Hardy-Weinberg equilibrium. Clinical and laboratory characteristics did not differ between genotypic groups. There was no statistically significant difference between controls and CHF patients for these genotype frequencies. In the subgroup analysis, there was also no statistically significant difference between ischemic and DCMP groups for genotype frequencies, with the exception of AT1R gene (Table II).

Endothelial Function Assessment

The flow-mediated arterial dilatation (EDD) among subjects with CHF was 9±5%, which was significantly lower than that in the healthy subjects (16±7%, $p < 0.001$, Figure 1). Also the nitroglycerin-induced arterial dilatation (EID) was lower in the CHF patients (13±6% vs 19±8%, $p = 0.001$, Figure 1). Comparison of ischemic with DCMP patients revealed that EDD (7±4% vs 12±6%, $p = 0.005$) was significantly impaired in the first group, but not EID (12±5% vs 14±7%, $p = 0.09$) (Figure 1). Moreover, EDD and EID were corre-

Table II. Polymophisms frequencies in the study population.

Characteristics	Controls group (n = 104)	CHF group (n=104)	p1	DCMP group (n=36)	Ischemic CHF group (n=68)	p2
ACE gene no. (%)						
II	16 (15)	19 (18)		8 (22)	11 (16)	
ID	47 (45)	47 (45)	0.8	17 (47)	30 (44)	0.6
DD	41 (40)	38 (37)		11 (31)	27 (40)	
ecNOS gene no. (%)						
aa	1 (1)	1 (1)		1 (3)	0 (0)	
ab	17 (16)	20 (19)	0.8	7 (19)	13 (19)	0.4
bb	86 (83)	83 (80)		28 (78)	55 (81)	
AT1R gene no. (%)						
AA	81 (78)	76 (73)		36 (100)	40 (59)	
AC	22 (21)	26 (25)	0.6	0 (0)	26 (38)	< 0.01
CC	1 (1)	2 (2)		0 (0)	2 (3)	

CHF = congestive heart failure. DCMP = dilated cardiomyopathy. ecNOS = endothelial constitutive NO synthase. ACE = angiotensin-converting enzyme. AT1R = angiotensin II type I receptor.

lated with age (r = -0.39, $p < 0.001$; r = -0.47, $p < 0.001$, respectively) and hemoglobin levels (r = -0.21, $p = 0.037$; r = -0.23, $p = 0.02$, respectively).

Gene Polymorphisms and Endothelial Function

Endothelial-Dependent Vasodilatation

In our study population, any relationship was found between EDD and ACE gene polymorphism (Figures 2 and 3). In the control group, also ecNOS and AT1R gene polymorphisms did not influence EDD (respectively, 19+9% in a carriers vs 16±7% in bb homozygotes, $p = 0.2$ and 16±5% in C carriers vs 17±8% in AA homozygotes, $p = 0.7$; Figure 2). Contrary in the CHF group we found a significant influence of ecNOS and AT1R polymorphisms on EDD. Particularly a carriers of ecNOS and C carriers of AT1R showed a lower EDD than bb and AA patients (respectively, 5±3% vs 10±6%, $p = 0.003$ and 5±4% vs 10±5%, $p = 0.03$; Figure 3).

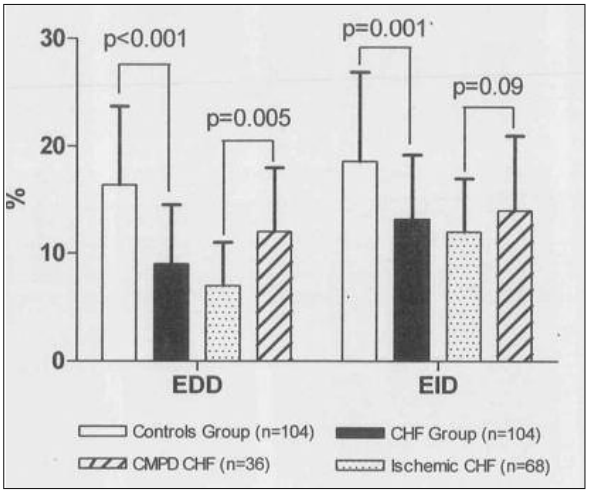

Figure 1. Endothelial-dependent and endothelial-independent vasodilatation in the study population group. EDD: Endothelial-dependent vasodilatation. EID: Endothelial-independent vasodilatation. CHF: congestive heart failure. DCMP: dilated cardiomyopathy.

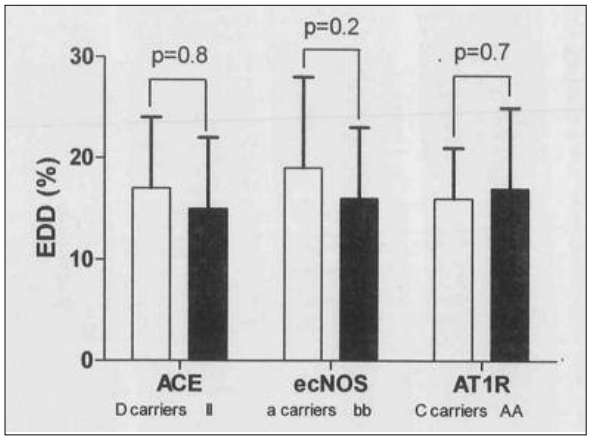

Figure 2. Endothelial-dependent vasodilatation in the control group stratified in according to gene polymorphisms investigated. EDD: Endothelial-dependent vasodilatation. ecNOS: endothelial constitutive NO synthase. ACE: angiotensin-converting enzyme. AT1R: angiotensin II type I receptor.

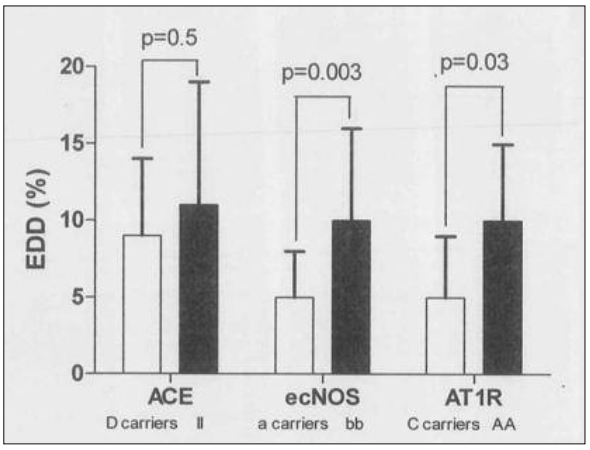

Figure 3. Endothelial-dependent vasodilatation in the CHF group stratified in according to gene polymorphisms investigated. EDD: Endothelial-dependent vasodilatation. ecNOS: endothelial constitutive NO synthase. ACE: angiotensin-converting enzyme. AT1R: angiotensin II type I receptor.

Endothelial-Independent Vasodilatation

Our analysis failed to demonstrate a relationship between EID and gene polymorphisms investigated (data not shown).

Determinants of Endothelial Function

When factors found significant for EDD were included in multiple regression test (hemoglobin level, age, presence of CHF, AT1R and ecNOS gene) only hemoglobin levels, age and presence of CHF were retained as significant (Multiple R = 0.567, adjusted R^2 = 0.30, F = 18.12, $p < 0.001$). Only age and hemoglobin levels were significantly associated with EID in multiple regression test (Multiple R = 0.578, adjusted R^2 = 0.32, F=28.96, $p < 0.001$).

Discussion

The healthy vascular endothelium exerts atheroprotective actions through vasoactive mediators such as nitric oxide (NO) and prostacyclin. There is evidence that as the endothelium ages, it is exposed to the damaging effects of environmental factors (hypertension, cigarette smoke, dyslipidemia, diabetes mellitus) and these protective properties diminish leading to a state of endothelial dysfunction. Endothelial dysfunction correlates with cardiovascular risk factors, and may be predictive of incident cardiovascular events. There have been few studies evaluating the heritability of endothelial function. Therefore, individual differences in endothelial function and, hence, susceptibility to later atherosclerosis, might relate not only to different levels of exposure to risk factors but also to inter-individual differences in the carriage of risk alleles of genes expressed in the vascular endothelium.

In the present study two major findings were found:

1. CHF patients had severe endothelial dysfunction discernible despite optimal medical treatment.
2. Endothelial dysfunction, measured as EDD, was partially determined by ecNOS and AT1R gene polymorphisms.

In the present study the endothelial function was impaired in CHF patients. EDD was impaired as was EID. Of note, we found that in ischemic CHF patients the endothelial dysfunction, measured as EDD, was deteriorated to a greater extent than smooth muscle function, measured as EID, as compared to non ischemic CHF patients.

Previously has been reported that a allele of ecNOS4 polymorphism is associated with coronary spasm, lower endothelial NO production and differential response of blood pressure to physical activity level[14]. The ACE I/D polymorphism plays a modifying role in the development of heart failure in hypertensive subjects[15] and has been associated with an increased risk of developing CAD[16] and with a more severe condition in Chinese patients with CHF[17]. Depending on salt intake, the AT1R 1166 CC genotype may or may not be associated with a tendency for volume expansion[18]. In the current investigation the ACE, ecNOS and AT1R gene polymorphisms were not significantly more prevalent among controls than among patients with CHF. Probably, the ethnicity (Turkish population) could have influence our findings. We found only a statistically significant difference in the AT1R gene between ischemic and DCMP patients, but the sample size was too small to obtain an affordable and definitive estimation.

To the best of our knowledge very limited data are available on the relationship between ecNOS, AT1R and ACE genes and endothelial function in CHF patients. We found that ecNOS and AT1R polymorphisms influence the EDD, but not EID. The molecular mechanism by which the genes interact with endothelial function in CHF patients is not known. Possible mechanisms are as follows: reduced intracellular availability of L-

arginine, reduced expression and activity of the nitric oxide system, increased ACE activity with decreased bradykinin and nitric oxide release, increased generation of superoxide anions, elevated levels of TNF-a with instability of NOS III (inducible) mRNA.

However, the main causes of the variability in EDD were age, hemoglobin levels and the presence of CHF. It appeared that these factors explained approximately 30% of the variability seen in EDD. These findings were not unexpected because advanced age was always found to impair EDD and hemoglobin inactivates nitric oxide by binding to guanylate cyclase.

Previously has been demonstrate that drug therapy, particularly ACE inhibitors and statins, improves EDD, but, even under optimal drug therapy used in our study, the effect of CHF on the endothelial function was not reversed.

The sample size of our study does not allow an affordable estimation of difference in the genotype distribution of polymorphisms investigated between CHF patients and healthy controls. In particular, in order to obtain a reliable estimate, a larger study population is clearly in demand. Additional clinical investigations are also needed to understand if exists a link between gene polymorphisms and medical therapy, potential target for pharmacogenetic strategies.

Conclusions

We found that endothelial dysfunction was present in CHF patients and that was partially predicted by ecNOS and AT1R gene polymorphisms. Further investigations are needed to better understand the molecular mechanisms and the clinical implications, particularly to assess whether patients with specific gene polymorphisms would benefit from a more aggressive medical and interventional management.

Conflict of Interest

I (we) certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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