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A.N. Parkhomenko, Ya.M. Lutay, V.E. Dosenko, V.L. Gurianova, A.A. Moibenko, A.A. Skarzevskiy

Influence of endothelial nitric oxide synthase $T^{-786}\rightarrow C$-promoter polymorphism on integral parameters of functional condition of arterial vessels

Наведено результати визначення частоти $T^{-786}\rightarrow C$-поліморфізму промотору гена ендотеліальної NO-синтази (eNOS) у 325 хворих із гострим коронарним синдромом і 104 практично здорових осіб та дані про вплив вказаного поліморфізму на розвиток реактивної гіперемії та жорсткість судинної стінки. Встановлено, що співвідношення гомозиготних носіїв генотипу T/T, гетерозигот із генотипом T/C і гомозиготних носіїв генотипу C/C становить 42,5, 41,2 та 16,3 % відповідно (в контролі – 40,4, 53,8 та 5,8 %; $P<0,01$ за χ^2-критерієм). Більш високий ступінь приросту діаметра плечової артерії встановлений у хворих з генотипом T/T: 8,03 % ± 0,71 % порівняно з 5,55 % ± 0,92 % при T/C-генотипі ($P<0,05$) та 5,30 % ± 1,21 % при C/C-генотипі ($P<0,05$). Швидкість розповсюдження пульсової хвилі на ділянках каротидно-променевої та каротидно-стегнової артерії також залежить від генотипу хворого: за генотипу T/T вона становить 9,10 ± 0,15 та 8,68 ± 0,26 відповідно, за T/C – 9,38 ± 0,18 та 8,74 ± 0,21, а за C/C – 9,71±0,22 та 10,02 ± 0,71 ($P<0,05$). Таким чином, отримані результати свідчать про суттєвий вплив $T^{-786}\rightarrow C$-поліморфізму промотору гена eNOS на інтегральні параметри функціонального стану артеріальних судин.

Ключові слова: алельний поліморфізм, ендотеліальна NO-синтаза, ендотеліальна дисфункція, швидкість розповсюдження пульсової хвилі, гострий коронарний синдром.

INTRODUCTION

Endothelium plays a crucial role in supporting of normal arteries functioning. Endothelium-derived relaxing and constricting factors control the vascular tone, endothelial and smooth muscle cells proliferation intensity, maintains antithrombogenic potential [2, 11, 23, 24, 25, 26, 27]. Nitric oxide (NO) produced by endothelial NO-synthase has the key significance in realization of protecting functions of endothelium [11, 13, 19, 24, 25, 26, 35]. As it was elucidated in some studies that SNP (single nucleotide polymorphism) $T^{-786}\rightarrow C$ in promoter of endothelial NO-syntase gene significantly affects the functioning of this system: replacement of thymidine by cytosine in -786 position of this gene promoter diminishes transcription activity and due to it decreases endothelial NO-synthase activity [9, 14, 30–32, 36, 38, 41]. However, while the effect of this SNP is elucidated on genetic level but the pathogenetic mechanisms of NOS3 gene variations in whole organism including figures of hemodynamic, functional condition of endothelium, reaction of vessel wall for the vasoactive agents application, others are not investigated enough. Only a few studies are dedicated to this problem. Yoshimura M. et al. [39] ascertained that SNP $T^{-786}\rightarrow C$ in promoter of NOS3 gene results in basal tone augmentation and more marked vasodilatation of coronary arteries in response to acetylcholine or isosorbide administration. Contrariwise in work of Erbs S. et al. an opposite evidence is presented that carriers of rare promoter gen-

otype variant (C/C genotype) have for 44% lower frequency of coronary and mammary arteries dilatation in response to acetylcholine administration (doppler velosimetry data) [16]. Taking into account presented intelligence and our own data about association of SNP $T^{-786}\rightarrow C$ in promoter of NOS3 gene with probability of acute coronary syndrome appearance [10, 12], it was aimed to determine the influence of this SNP upon the integral features of arterial vessels state using the test for reactive hyperemia and detecting blood vessel wall stiffness performing ultrasonic estimate of pulse wave arrival frequency.

METHODS

Into the study were taken the patients hospitalized to the Reanimation and Intensive Care department of National Scientific Center «M.D. Strazhesko Institute of Cardiology", Medical Academy of Science (Ukraine) with diagnosis unstable angina pectoris (USP) or acute myocardial infarction (AMI). 325 patients suffering from acute coronary syndromes (ACS) were examined (113 patients without ST segment elevation and 212 patients with ST segment elevation electrocardiographically). USP and AMI diagnosed basing on clinical, electrocardiographic and biochemical examinations according to existing recommendations [3, 4]. Patients with IIB – III stages of chronic heart failure, true cardiogenic shock, severe form of diabetes mellitus, marked renal and hepatic failure, bronchial asthma, homeostatic imbalances, acute brain circulatory injury, traumas and vast surgical interventions, acute and exacerbations of chronic inflammatory processes, oncologic and systemic diseases were not taken into the study. Control group includes 104 practically healthy donors without cardiovascular pathologies basing on anamnesis data, electrocardiographic estimation blood pressure measurement. Control group and group of patients are not different by age and sex structure ($P>0.05$ by χ^2-test).

Venous blood sampling for genotyping was performed in sterile conditions to 2,7 mL monovetts with potassium salt of ethylenediaminetetraacetic acid as anticoagulant ("Sarstedt", Germany), then frozen and saved by the temperature -20°C. DNA was extracted from the whole blood using kit Isogene (Russian Federation). SNP $T^{-786}\rightarrow C$ in promoter of NOS3 gene was determined using polymerize chain reaction with restriction fragments length analysis [17] and polymerize chain reaction in real time using Custom Taq-Man SNP Genotyping Assay (Applied Biosystems, USA), including upstream (5`-CCACCAGGGCATCAAGCT-3`), downstream (5`-GCAGGTCAGCAGAGAGA-CTAG-3`) primers and fluorescent probes to T allele (VIC-TTCCCTGGCTGGCTGA-NFQ) and to C-allele (FAM-CCTGGCCGGCTGA-NFQ).

Test for reactive hyperemia was performed using the apparatus for ultrasonic diagnostics SonoAce PICO (Medison, Korea) by vessel sensor HL5-9ED (7,5 MHz/40 mm). Nitrates (excluding medications with short time of action for rapid relief of angina pectoris attacks) were abolished for patients 24 hours prior to the test performing. The test was carried out in the morning on an empty stomach after 10 minutes of rest decubitus. After the initial diameter of humeral artery measurement (average values of 4 measurements with 1 cm interval between the points) the cuff of monometer put on patient's humerus upper the site of measurement and pressure 200 mm Hg was pumped and held for 5 minutes. In 80 seconds after decompression the second measurement of artery diameter and index of endothelium dependent vasodilatation was calculated as percent of artery diameter increase comparing with initial measurement [8, 15]. The test with reactive hyperemia was performed in 63 patients.

The blood vessel wall stiffness was estimated performing ultrasonic detection of pulse wave arrival frequency by apparatus Complior® SP (ARTECH medical, France) [5].

Statistical analysis of results was performed using "MicrosoftTExcel 2000" and

statistical programs SPSS (12 version, USA). Distribution significance was estimated by χ^2-test and Student's t-test, values of $P<0.05$ accepted as significant.

RESULTS AND DISCUSSION

Genotyping of patients for SNP $T^{-786}\rightarrow C$ in promoter of endothelial NO-synthase gene resulted in such genotypes distribution: -786T/T, -786T/C and -786C/C: 42,5%, 41,2% and 16,3% in patients suffering from ACS 48,2%, 45,8% and 6,0% in control group correspondingly (table 1). Determined distribution of different genotypes frequencies in case and control groups is corresponding to Hardy–Weinberg equilibrium.

Consequently, SNP in promoter of endothelial NO-synthase gene in position -768 was detected in 57.5% patients with ACS, and 16.3% of them were homozygous carriers of -786C/C genotype. While there were not differences of T/T and T/C genotypes frequencies between two groups but probability of C/C promoter genotype variant was significantly higher in group of patients with ACS compared with control group (16,3% in patients with ACS group to 5,8% in control group, $P<0.01$). Among the patients with ACS without ST segment elevation four times more frequently than in control group (22.1 to 5.8%, $P<0.01$) and twice more frequently than among the patients with ST segment elevation (22.1 to 13.2%, $P<0.05$) the homozygous carriers of C/C eNOS gene promoter genotype variant were revealed. Findings let to draw a conclusion about this SNP influence exerting on acute forms of coronary heart disease (CHD) development probability, especially in patients cardiographically without stable ST segment elevation.

Meta-analysis data show that significant differences by carriers of homozygous C/C eNOS gene promoter genotype variant frequency were observed among the healthy population between several ethnic groups (1.1 % – for Asian, 15.36 % – for non-Asian groups, $P<0.0001$) [6]. Literature evidences about the role of this SNP in development of CHD and its acute forms are contradictory [1, 7, 17, 22, 29, 30, 34, 36, 39]. Populational study performed in 9 several regions of Great Britain showed that by of SNP $T^{-786}\rightarrow C$ in promoter of NOS3 gene analysis the distribution of homozygous by frequent allele, heterozygous and homozygous by rare allele carriers was 37.7; 47.8 and 14.5 %, correspondingly. 2965 patients were under observation for 8 years and it permitted to elucidate the SNP in promoter of NOS3 gene influence on frequency of CHD development [22]. Occurrence of T/T, T/C, C/C variants in -786 position of promoter among Italians is approximate like it is in Great Britain and Ukraine [10, 17, 32]. Further to it the risk of CHD was significantly higher among homozygous carriers of C/C allele variant compared to persons homozygous by T/T allele ($P<0.01$), and C/C genotype was independent risk factor for development of coronary atherosclerosis [17]. It was shown that itself rare allele C presence is a risk factor for CHD ($P=0.02$ compared to T/T genotype) and among patients suffering from it carriers of rare allele had more pronounced atherosclerotic injury of coronary

Table 1. SNP $T^{-786}\rightarrow C$ in promoter of endothelial NO-synthase gene genotype variants distribution in patients with ACS and healthy donors (%)

Genotype	Control (n=104)	ACS (n=325)	ACS without segment ST elevation (n=113)	ACS with segment ST elevation (n=212)
TT	40.4	42.5	42.5	42.5
TC	53.8	41.2	35.4	44.3
CC	5.8	16.3**	22.1**	13.2*

* P<0,05 compared to control group

** P<0,01 compared to control group

arteries according to coronarographic data [17]. In Ghilardi G. et al. study it was shown that among patients operated on for internal carotid artery stenosis C/C genotype found twice more often in comparison with control group (26 and 13 % correspondingly, P=0.08) and within group of patients with internal carotid artery stenosis C/C genotype was more frequently in group with ulcered atherosclerotic plaque (44 and 17 % correspondingly, P=0.003) [17]. Into Hyndman M.E. et al. research carried out in Canada were included 705 male persons without CHD in anamnesis [20]. Distribution of different eNOS gene promoter genotype variants (T/T, T/C, C/C) was closed to the same in Caucasians: 38.9; 46.1 and 15.0 %. In individuals carrying C/C genotype noted significantly higher level of systolic blood pressure and more often arterial hypertension was diagnosed than in others. It permitted authors to conclude that C/C NOS3 gene promoter genotype is a factor predisposing to arterial hypertension. Iwai N. et al. showed that in Japanese population frequency of C allele is quite low (20.2 % of whole population), and frequency of homozygous carriers of rare allele (C/C) is approximately 1 % of population [21]. In patients with AMI several promoter variants were not different from the same in whole population and it lets to conclude that this SNP does not play any role in AMI pathogenesis in Japanese population [37]. Contrarily in research of Nakayama M. et al. SNP $T^{-786}\rightarrow C$ in promoter of NOS3 gene was associated with coronary spasm and more frequently was revealed in patients suffering from AMI especially without coronary organic stenosis by angiographic results [30, 31].

Comparing several populations of almost healthy people the interesting date were obtained. So rare genotype C/C in promoter of NOS3 gene frequency is 5,8 % among healthy donors in Ukraine and that is significantly higher than in Japanese population and lower compared with Caucasians (Italian, English, Spanish, French populations), (Table 2).

To introduce clarity into knowledge of

Table 2. Comparative analysis of SNP $T^{-786}\rightarrow C$ in promoter of endothelial NO-syntase gene frequency distribution among healthy persons in several ethnic groups (n (%))

Genotype	Пархоменко А.Н. и др.	Colombo M. et al., 2003 [7]	Ghilardi G. et al., 2002 [17]	Jeerooburkhan N. et al., 2001 [22]	Alvarez R. et al., 2001 [1]	Poirier O. et al., 1999 [32]	Poirier O. et al., 1999 [32]	HyndmanM.E. et al., 2002 [20]	Iwai N. et al., 1999 [21]
	Ukraine (n=104)	Italy (n=138)	Italy (n=133)	Great Britain (n=272)	Spain (n=300)	Northern Ireland (n=155)	France (n=421)	Canada (n=705)	Japan (n=3918)
T/T	42 (40.4)	47 (34.1)	54 (40.6)	1026 (37.7)	120 (40)	57 (36.8)	126 (29.9)	274 (38.9)	3127 (79.8)
T/C	56 (53.8)	71 (51.4)	61 (45.9)	1300 (47.8)	137 (45.7)	64 (41.3)	220 (52.3)	325 (46.1)	748 (19.1)
C/C	6 (5.8)	20 (14.5)	18 (13.5)	394 (14.5)	43 (14.3)	34 (21.9)	75 (17.8)	106 (15.0)	43 (1.1)

changed genotype mechanisms in pathogenesis of CHD and ACS particularly we estimated endothelial dysfunction intensity and level of vessel wall stiffness in part of examined patients.

By results of cuff test with reactive hyperemia carried out for 63 examined patients with ACS (distribution of eNOS gene promoter genotype variants was 46.0, 36.5, 17.5% and was not differed from control group), significantly higher level of humeral artery diameter increase was observed in patients carrying -786T/T genotype (8.03 ± 0.71% in comparing with 5.55 ± 0.92 in patients with T/C genotype (P<0.05) and 5.30 1.21 in patients with C/C genotype (P<0.05)). Consequently, SNP in NO-syntase gene is associated with decrease of endothelium dependent vasodilatation after cuff test that can be evidence of more marked endothelial dysfunction in this category of patients (Fig. 1).

Vessel wall stiffness was determined in 73 patients with ACS by measurement of pulse wave arrival frequency (PWAF) along the carotid-femoral and carotid-radial arteries segments using non-invasive method. Primary clinical and anamnestic characteristics and distribution of several genotype variants in eNOS gene promoter of this access also was corresponded to the general whole of examined patients. PWAF on carotid-radial arteries segment to a great extent is figure of stiffness changes in arteries of muscular type and on carotid-femoral arteries segment – in arteries of elastic type.

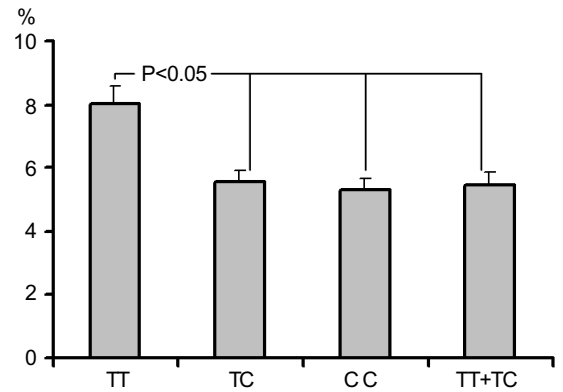

Figure 1. Results of cuff test with reactive hyperemia in patients with ACS carrying several genotypes of SNP $T^{-786}\rightarrow C$ in promoter of NOS3 gene

Patients carrying C/C genotype of NOS3 gene promoter were characterized by significantly higher PWAF along both carotid-radial arteries and carotid-femoral arteries segments compared to the same in patients carrying T/T genotype. In patients carrying genotype homozygous by frequent allele (-786T/T) and heterozygous (-786T/C) was not differed but summary carriers of rare changed allele C (-786T/C and -786 C/C) had higher PWAF along carotid-radial arteries.

Thus, in our study was found that SNP in promoter of endothelial NO-synthase gene is associated with decreased endothelium dependent vasodilatation after cuff test verifying presence of endothelial dysfunction in this category of patients. Also we for the first time demonstrated interrelation between SNP in eNOS gene and stiffness of elastic and muscle type arteries. At present time this figure is used as integral marker of vessel wall condition, thus its augmentation in patients with SNP in NOS3 gene is evidence of more marked and prolonged organic and functional changes in vascular system in this category of patients. Clinically these changes can be realized as coronary arteries tone augmenta-

Table 3. Pulse wave arrival frequency (PWAF) along the carotid-femoral and carotid-radial arteries segments subject to genotype in patients with ACS

Pulse wave arrival frequency, conv.units	T/T (n=30)	T/C (n=34)	C/C (n=9)	T/C+C/C (n=43)
Carotid-radial arteries	9.10±0.15	9.38±0.18	9.71±0.22*	9.45±0.15*
Carotid-femoral arteries	8.68±0.26	8.74±0.21	10.02±0.71*	9.01±0.23

* P<0.05 in comparing to patients carrying T/T genotype.

tion, increased inclination to coronary spasm and perverted reaction of coronary arteries to acetylcholine administration, higher restenosis risk after coronary stent placement that is elucidated in several articles [18, 28, 29, 34, 39, 40]. The referred literature date and our study results acknowledge the more marked vessel dysfunction in patients carrying rare allele of NOS3 gene and permit to define the category of patients with high risk of cardiovascular complications.

А.Н. Пархоменко, Я.М. Лутай, В.Е. Досенко, А.А. Мойбенко, А.А. Скаржевский

ВЛИЯНИЕ $T^{-786}\rightarrow C$-ПОЛИМОРФИЗМА ПРОМОТОРА ГЕНА ЭНДОТЕЛИАЛЬНОЙ NO-СИНТАЗЫ НА ИНТЕГРАЛЬНЫЕ ПАРАМЕТРЫ ФУНКЦИОНАЛЬНОГО СОСТОЯНИЯ АРТЕРИАЛЬНЫХ СОСУДОВ

Приводятся результаты определения частоты $T^{-786}\rightarrow C$-полиморфизма промотора гена эндотелиальной NO-синтазы (eNOS) у 325 больных с острым коронарным синдромом и 104 практически здоровых людей, а также данные о влиянии указанного полиморфизма на развитие реактивной гиперемии и жесткость сосудистой стенки. Установлено, что соотношение гомозиготных носителей генотипа T/T, гетерозигот с генотипом T/C и гомозиготных носителей генотипа C/C составляет 42,5, 41,2 та 16,3 % соответственно (в контроле – 40,4, 53,8 и 5,8 %; $P<0.01$ по χ^2-критерию). Более высокая степень прироста диаметра плечевой артерии установлена у больных с генотипом T/T: 8,03 % ± 0,71% по сравнению с 5,55 % ± 0,92 % при T/C-генотипе ($P<0,05$) и 5,30 ± 1,21 % при C/C-генотипе ($P<0,05$). Скорость распространения пульсовой волны на участках каротидно-лучевая и каротидно-бедренная артерии также зависит от генотипа больного: при генотипе T/T она составляет 9,10 ± 0,15 и 8,68 ± 0,26 соответственно, при T/C – 9,38 ± 0,18 и 8,74 ± 0,21, а при C/C – 9,71 ± 0,22 и 10,02 0,71 ($P<0,05$). Таким образом, полученные результаты свидетельствуют о существенном влиянии $T^{-786}\rightarrow C$-полиморфизма промотора гена eNOS на интегральные параметры функционального состояния артериальных сосудов. Ключевые слова: аллельный полиморфизм, эндотелиальная NO-синтаза, эндотелиальная дисфункция, скорость распространения пульсовой волны, острый коронарный синдром.

ННЦ «Ин-т кардиологии им. акад. Н.Д. Стражеско» АМН Украины, Киев;

Ин-т физиологии им. А.А. Богомольца НАН Украины, Киев

A.N. Parkhomenko, Ya.M. Lutay, V.E. Dosenko, V.L. Gurianova, A.A. Moibenko, A.Skarzevskiy

INFLUENCE OF ENDOTHELIAL NITRIC OXIDE SYNTHASE $T^{-786}\rightarrow C$ PROMOTER POLYMORPHISM ON INTEGRAL PARAMETERS OF FUNCTIONAL CONDITION OF ARTERIAL VESSELS

Frequency of eNOS $T^{-786}\rightarrow C$ promoter polymorphism in 325 patients with acute coronary syndrome and 104 control persons and influence of this polymorphism on reactive hyperemia and arterial stiffness were determined. Interrelation between T/T homozygous, T/C heterozygous and C/C homozygous was 42,5%, 41,2% and 16,3%, correspondingly (in control – 40,4%, 53,8% and 5,8%; $P<0.01$ by χ^2-test). Higher degree of brachial arteries diameter increase in response to ischemia was estimated in patients with T/T genotype: 8,03 ± 0,71% compared to 5,55 ± 0,92 % in T/C ($P<0.05$) and 5,30 ± 1,21 % in C/C genotypes ($P<0.05$). Speed of pulse wave spreading on carotid-radial and carotid-femoral arteries segments was also depended on patients genotype: in T/T genotype was 9,10 ± 0,15 and 8,68 ± 0,26 correspondingly, in T/C - 9,38 ± 0,18 and 8,74 ± 0,21, and in C/C carriers - 9,71 ± 0,22 and 10,02 ± 0,71 ($P<0.05$). Thus, the data obtained indicate on significant influence of $T^{-786}\rightarrow C$ polymorphism on integral parameters of functional condition of arterial vessels. Key words: allelic polymorphism, endothelial NO-synthase, endothelial dysfunction, speed of pulse wave arrival frequency, acute coronary syndrome.

National Scientific Center «M.D. Strazhesko Institute of Cardiology", Medical Academy of Sciences of Ukraine, Kyiv; O.O.Bogomoletz Institute of Physiology, National Academy of Sciences of Ukraine, Kyiv

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National Scientific Center «M.D. Strazhesko Institute of Cardiology", Medical Academy of Sciences of Ukraine, Kyiv;
O.O. Bogomoletz Institute of Physiology, National Academy of Sciences of Ukraine, Kyiv
dosenko@biph.kiev.ua

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