

Biomarker-based Prognostication of Adverse Cardiac Remodeling after STEMI: the Role of Single Nucleotide Polymorphism T786C in Endothelial NO-synthase gene

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ABSTRACT

BACKGROUND: Endothelial NO-synthase (eNOS) is constitutive enzyme, which expresses in mature endothelial cells and promotes direct vascular dilatation. Single nucleotide polymorphism (SNP) of T786C in eNOS gene may influence on adverse cardiac remodeling

after ST-elevation myocardial infarction (STEMI). Purpose of the study was to investigate possible associations between SNP T786C in eNOS gene and adverse cardiac remodeling after STEMI.

METHODS: 177 acute STEMI patients treated with percutaneous coronary intervention and thrombolysis that were admitted to intensive care unit of GI "L.T.Malaya TNI NAMSU" were enrolled in the study. Anthropometry, cardiovascular risk assay, coronary angiography, echocardiography and biomarkers' measure were performed at baseline. The DNA extraction was performed with a commercial kit using real-time polymerase chain reaction PCR.

RESULTS: There were correlations between 786CC polymorphism in eNOS gene and adverse cardiac remodeling ($r = 0.48$; $p = 0.001$), LDL cholesterol ($r = 0.32$; $p = 0.012$), type 2 diabetes mellitus ($r = 0.30$; $p = 0.042$), diastolic BP ($r = -0.26$; $p = 0.048$), unstable angina prior to STEMI ($r = 0.25$; $p = 0.047$) and total quantity of complicated STEMI ($r = 0.23$; $p = 0.042$). Additionally, there were not significant relations between 786CC polymorphism in eNOS gene and multiple coronary vessel injury, STEMI localization, levels of circulating biomarkers of myocardial injury, and amount of damaged coronary arteries. Using univariate and multivariate regressive logistic analysis we found that 786CC genotype of eNOS was independent predictor for late adverse LV remodeling (β -coefficient = 1.57342; odds ration = 4.8231; 95% confidence interval = 1.5349-15.1552; $p = 0.0071$).

CONCLUSION: The polymorphism 786CC in eNOS gene was found as an independent predictor for late adverse cardiac remodeling after STEMI.

Key words: STEMI; Single nucleotide polymorphism T786C; Endothelial NO-synthase gene; Adverse cardiac remodeling

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INTRODUCTION

Nitric oxide (NO) is well established factor that directly promotes vascular dilatation, regulates vascular smooth muscle cell proliferation and migration, endothelial permeability and has indirect antithrombotic effects^[1]. NO is synthesized in vascular endothelium from its precursor L-arginine with endothelial NO synthase (eNOs)^[2]. Endothelium-derived NOs appears to be a constitutive enzyme, expression of which is depended on appropriate eNOs gene^[3]. Previous preclinical and clinical studies have been shown that genetic deletion of promoter region of eNOs gene – single nucleotide polymorphisms (SNP) T786C - frequently associated with lowered NO production^[4]. SNP T786C in eNOs gene corresponded to lipid infiltration of vascular wall, apoptosis of mature and progenitor endothelial cells, oxidative stress-induced microvascular inflammation, mononuclear infiltration, proliferation of vascular smooth muscle cells (VSMCs), accumulation of extracellular matrix components, and platelet aggregation on the surface of endothelium^[5-7]. Collectively, impaired eNO bioavailability was found as main trigger for endothelial dysfunction, accelerating atherosclerosis, and thrombosis^[8]. Additionally, deficiency of eNO through activating oxidant-sensitive pro-inflammatory cellular transcription factors, such as nuclear factor κ B (NF κ B) and FOXO-3, attenuate oxidative stress, and mitochondrial dysfunction that are considered as a main factor contributing in apoptosis and necrosis^[9]. Indeed, altered suppressive effect due to eNO deficiency influenced on proliferation of VSMCs, declined production of vascular endothelial growth factor-A and accumulation of inflammatory / immune cells, which exacerbate dysfunctional endothelium, persistence cell injury and modulate vessel stenosis and intravascular thrombus^[10]. On the other hand, restored eNOs activity and improved NO production was able to inhibited cardiac myocyte apoptosis and promoted cardiac function after STEMI^[11]. Because all these factors play a pivotal role in pathogenesis of adverse cardiac remodeling after successful opening of coronary artery with percutaneous coronary intervention (PCI) due to ST-elevation myocardial infarction (STEMI)^[12], and SNP N786C in eNOs gene, a G to T substitution at nucleotide 894 in exon 7 of eNOS, was found to be associated with STEMI^[13], we have been hypothesized that this polymorphism could be a predictor of adverse cardiac remodeling. Previous studies have shown that T-786C Glu298Asp polymorphism in eNOs gene has yielded conflicting associations with cardiovascular and renal diseases in numerous general populations^[14-16], but adverse cardiac remodeling was not being investigated in this conclusion. The aim of the study was to investigate possible associations between SNP T786C in eNOS gene and adverse cardiac remodeling after STEMI.

METHODS

Patients' population

A total of 268 patients with confirmed acute STEMI were analyzed for participation in the study. From entire population of STEMI ($n = 268$) according to inclusion and non-inclusion criteria it was enrolled 177 individuals that were admitted to intensive care unit of GI "L.T.Malaya TNI NAMSU" with acute STEMI during 2-12 hours of symptoms onset in a given period between August 2016 and July 2018. STEMI was diagnosed according to ECS Guidelines (2017)^[5]. Inclusion criteria were: confirmed STEMI, age > 18 years old, and lack of contraindication to PCI. Non-inclusion criteria were previous myocardial infarction, established chronic heart failure, known malignancy, severe comorbidities (anemia,

chronic obstructive lung disease, bronchial asthma, liver cirrhosis, chronic kidney disease, valvular heart disease, bleeding), inability to understand of written informed consent. Primary PCI with bare-metal stent (COMMANDER, "Alvimedica", Turkey) implantation was performed in 133 patients and 44 patients were previously treated with primary thrombolysis (tenecteplase, alteplase) before admission with followed PCI during 6-12 hours after initial STEMI confirmation. Thrombolysis was done with tenecteplase (Metalise, Boehringer Ingelheim Pharma, Germany), depending on patients weight and was not more then 50 mg iv bolus. Alteplase (Actilyse, Boehringer Ingelheim Pharma, Germany) 100mg infused intravenous during 2 hours. All investigated patients received adjuvant treatment due to current ESC recommendations^[17].

Ethical declaration

All procedures performed in the study involving human participants were in accordance with the ethical standards and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards and approved by the local ethics committee (Protocol №8, 29.08.2016). Inform consent was obtained from each patient.

Adverse LV remodeling determination

The study patients were followed through the outpatient clinic of our institution at 6-month observation. Adverse cardiac remodeling was defined as increased LVEDV (>10% from baseline)^[18].

Coronary angiography

Conventional coronary angiography was performed immediately after admission of the patients to the hospital using Digital X-Ray system "Integris Allura" (Philips Healthcare, Best, The Netherlands) and managed by radial or femoral vascular access. Coronary arteries were visualized with two-to-three orthogonal projections per conventional protocol. In this study the contrast "Ultravist-370" (Baier Pharma GmbH, Germany) and automatic contrast injector were used. The contrast amount used in coronary angiography in each injection was 8 - 10 mL at 4 mL/s for the left coronary artery and 6 mL at 3 mL/s for the right coronary artery (radiation exposure 20 to 35 mGycm). The number of views obtained was decided by the operator depending on coronary anatomy. The coronary arteries were divided into segments according to the American Heart Association classification^[19].

Determination of STEMI prognosis

TIMI score was used to validate prognostic capacity after STEMI^[20].

Determination of risk factors and comorbidities

Hypercholesterolemia (HCE) was diagnosed if total cholesterol (TC) level was above 5.2 mmol/L, and/or low density lipoprotein cholesterol (LDL) level was above 3.0 mmol/L, and/or level of triglycerides (TG) was above 1.7 mmol/L according to with European Cardiology Society dyslipidemia guideline (2016)^[21]. Hypertension was diagnosed if systolic blood pressure (SBP) was >140 mm Hg, and/or diastolic blood pressure (DBP) >90 mm Hg according to European guideline on diagnostics and treatment of arterial hypertension (2018)^[22]. Type 2 diabetes mellitus determined according to new ADA statement (2017)^[23]. Premature MI was verified if it appeared to be diagnosed in patients 55 years and younger.

Echo and Doppler examination

Echo-CG performed on "Aplio 500" (TUS-A500) TOSHIBA MEDICAL SYSTEMS CORPORATION with usage of 3.5 MHz phase probe at discharge and at 6-month observation period.

Left ventricular end diastolic volume (LV EDV), left ventricular end systolic volume (LV ESV), left ventricular ejection fraction (LVEF) measuring were done according to Simpson’s method per contemporary recommendation^[24]. Left ventricular myocardial mass (LVMM) was calculated in automatic manner per protocol of echocardiograms evaluation^[24].

Blood samples

Blood samples were drawn immediately before PCI and at 6 month of investigation. Blood samples were centrifuged, serum was isolated within 30 min of sample acquisition and after then they were freezed with -70°C and stored in plastic tubes until being shipped to the laboratory of immune-chemical and molecular-genetic researches of GI “L.T.Malaya TNI NAMSU”.

Troponin I (Tn I) level measuring performed with chemo luminescent immunoassay (Humalyser 2000, Mannheim, Germany). The TnI level average was 0.5-50 ng/mL.

Total cholesterol (TC), low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol and triglycerides (TG) were measured direct enzymatic method (Roche P800 analyzer, Basel, Switzerland). The intra-assay and inter-assay coefficients of variation were < 5%.

Fasting glucose level was measured by a double-antibody sandwich immunoassay (Elecsys 1010 analyzer, F. Hoffmann-La Roche Diagnostics, Mannheim, Germany). The intra-assay and inter-assay coefficients of variation were < 5%.

Total creatine kinase (CK) and CK MB-fraction (CK-MB) were analyzed using immunoinhibition method on quantitative immunoassay analyzer Humalyser 2000 (HUMAN GmbH, Germany) according to the manufacturers’ recommendations.

N-terminal fragment of brain natriuretic peptide (NT-proBNP) was measured by commercially available standard kit (R&D Systems

GmbH, Wiesbaden-Nordenstadt, Germany). The NT-proBNP level average was 10-12000 pg/mL.

SNP T786C (rs2070744) in eNOS gene determination

The DNA extraction was performed according to the protocol for a commercial set «DNA-Sorb-B» (AmplySens, Russia). The assessment of allelic states of SNP studied was performed using real-time polymerase chain reaction (PCR). For real-time PCR a commercial kit qPCR mix “SNP-Screen», catalogue number NP-554-100 (Syntol, Russia) was used. PCR was performed on CFX96 thermocycler (BioRad, USA) using an allelic discrimination test. PCR conditions: 95°C – 3’, 40 cycles of 95°C – 15”, 65°C – 40”.

Statistics

Statistical analyses were performed using SPSS for Windows v.23 (USA). Continuous variables are presented as mean ± standard deviation (SD) when normally distributed, or median and interquartile range (IQR) if otherwise. Categorical variables are presented as frequencies (n) and percentages (%). Mann-Whitney and Wald-Wolfowitz criteria were used for intergroup differences and quantitative values. The qualitative variables are expressed as percentages, and were analyzed by the χ² and exact Fisher tests. The genotypic data were checked for Mendelian pedigree. Allele frequencies were estimated, and all polymorphisms were tested for Hardy–Weinberg Equilibrium. We preformed univariate and multiple variate log-regression analysis to determine the factors that could predict late adverse cardiac remodeling at 6 month and combined end point. We calculated beta coefficient, standard errors (SE), odds ratio (OR), 95% confidence interval (CI) for each factor. Factor, for which P values were calculated as >0.5 were not included in the multiple variate log-regression analysis. All differences were considered statistically significant with 2-tailed *p* < 0.05.

Table 1 Characteristics of STEMI patients included in the study depending on major / minor alleles

	Entire population (n = 177)	TT genotype (n = 73)	TC genotype (n = 64)	CC genotype (n = 40)	P value
		1	2	3	
Age, years (SD)	61.73 ± 9.44	59.00 ± 10.10	59.27 ± 9.92	58.53 ± 8.29	NS
Male, n (%)	139 (78.5%)	57 (78.1%)	50 (78.1%)	32 (80.0%)	NS
Female, n (%)	38 (21.5%)	16 (21.9%)	14 (21.9%)	8 (20.0%)	
Hypertension, n (%)	146 (82.5%)	60 (82.2%)	55 (85.9%)	31 (77.5%)	NS
T2DM, n (%)	44 (24.9%)	15 (20.5%)	13 (20.3%)	16 (40.0%)	PTT-CC =0.027; PTC-CC =0.029
Smoking, n (%)	84 (47.5%)	29 (39.7%)	31 (48.4%)	24 (60.0%)	PTT-CC =0.039
Stable CAD prior to STEMI, n (%)	107 (60.5%)	42 (57.5%)	39 (60.9%)	26 (65.0%)	NS
HCE, n (%)	105 (59.3%)	45 (61.6%)	37 (57.8%)	23 (57.5%)	NS
BMI > 30 кг/м2, n (%)	69 (39.0%)	29 (39.7%)	26 (40.6%)	14 (35.0%)	NS
Unstable angina prior to STEMI, n (%)	67 (37.9%)	21 (28.8%)	24 (37.5%)	22 (55.0%)	PTT-CC =0.011; PTC-CC =0.080
Previous MI, n (%)	31 (17.5%)	11 (15.1%)	7 (10.9%)	13 (32.5%)	PTT-TC =0.084
Premature MI, n (%)	74 (41.8%)	29 (39.7%)	21 (32.8%)	24 (60.0%)	PTT-CC =0.039; PTC-CC =0.007
Peak TnI, ng/mL	17.7 [6.34-77.2]	17.97 [9.73-60.5]	17.7 [3.87-129.0]	25.05 [3.99-180.0]	NS
Peak CK-MB, U/L	103.3 [44.9-28.95]	108.90 [74.30-328.40]	112.90 [55.00-196.90]	43.85 [30.20-158.50]	PTT-CC =0.064
NT-proBNP, pg/mL	246 [107 - 405]	247 [118 - 388]	253 [121 - 375]	241 [105 - 344]	NS
Total cholesterol, mmol / L	4.82 [3.95-5.63]	4.83 [3.91-5.79]	4.62 [3.95-5.50]	4.89 [3.79-5.58]	NS
HDL-cholesterol, mmol / L	1.12 [0.92-1.28]	1.12 [0.92-1.28]	1.13[0.94-1.31]	1.05 [0.90-1.28]	NS
LDL cholesterol, mmol / L	3,00 2.03-3.63]	2.64 [1.87-3.46]	2,79 [2.04-3.56]	3,36 [2.46-4.13]	PTT-CC =0.048

BMI: body mass index; CAD: coronary artery disease; T2DM: type 2 diabetes mellitus; HCE: hypercholesterolemia; MI: myocardial infarction; LDL: low-density lipoprotein; HDL: high-density lipoprotein; Tn: cardiac troponin; NT-proBNP: N-terminal fragment of pro-brain natriuretic peptide; CK-MB: creatin kinase MB fraction.

Table 2 STEMI localization and number of damaged coronary arteries in patients included in the study depending on major / minor alleles

	Entire population (n = 177)	TT genotype (n = 73)	TC genotype (n = 64)	CC genotype (n = 40)	P value
		1	2	3	
STEMI risk scoring					
TIMI risk score, point (IQR)	6 [4-7]	6 [5-7]	6 [4-7]	7 [5-8]	0.62
STEMI Localization					
Anterior LV wall, n (%)	97 (54.8%)	44 (60.3 %)	33 (51.6 %)	20(50.0 %)	NS
Posterior LV wall, n (%)	70 (39.5%)	29 (39.7 %)	31 (48.4 %)	20 (50.0 %)	
Amount of coronary vessels injured					
One artery, n (%)	53 (29.9%)	24 (32.9 %)	18 (33.3 %)	11 (33.3 %)	NS
Two and more arteries, n (%)	103 (58.2%)	45 (61.6 %)	36 (66.6 %)	22 (66.6 %)	NS
Left anterior descending, n (%)	47 (26.6%)	25 (34.2%)	9 (14.1%)	13 (32.5%)	PTT-TC =0.009; PTT-CC =0.048
Right coronary artery, n (%)	41 (23.2%)	22 (30.1%)	12 (18.8%)	7 (17.5%)	NS
Circumflex coronary artery, n (%)	20 (11.3%)	9 (12.3%)	6 (9.4%)	5 (12.5%)	NS
Left main, n (%)	12 (6.8%)	7(9.6%)	2 (3.1%)	3 (7.5%)	PTT-TC=0.035; PTT-CC=0.05
Complications of STEMI					
Total quantity of complicated STEMI, n (%)	69 (38.9%)	30 (41.1 %)	22 (34.4 %)	17 (42.5 %)	p1-2 = 0.419; p1-3 = 0.885; p2-3 = 0.405
II-III Killip class of HF, n (%)	28 (15.8%)	16 (21.9 %)	8 (12.5 %)	4 (10.0 %)	p1-2 = 0.148; p1-3 = 0.090; p2-3 = 0.698
IV Killip class of HF, n (%)	10 (0.6%)	5 (6.8 %)	1 (1.6 %)	4 (10 %)	PTC-CC = 0.071
Cardiac arrest, n (%)	3 (1.7%)	1 (1.4 %)	0	2 (5.0 %)	NS
Acute 2-3 grade av-block, n (%)	5 (2.8%)	2 (2.7 %)	1 (1.6 %)	2 (5.0 %)	NS
Recurrent angina	1 (0.6%)	1 (1.4 %)	0	0	NS
Adverse remodeling at 6 month, n (%)	46 (26.0%)	14 (19.2%)	17 (26.6%)	15 (37.5%)	PTT-CC = 0.033

AV: atrium-ventricular; HF: heart failure; MI: myocardial infarction; IQR: interquartile range.

RESULTS

Table 1 is reported characteristic of the STEMI patients included in the study. It turned out that T and C alleles were presented about 60% and 40% of entire cohort patients. Sorting of STEMI patients according to genotypes of T786C eNOs gene appeared to be shown following, such as TT genotype was found in 73 patients (42%), TC genotypes was identified in 64 patients (36%) and CC genotypes was determined in 40 patients (22%) respectively. All STEMI patients were completely matched each other to age, sex, hypertension, stable CAD prior to STEMI, hypercholesterolemia, and obesity. Consequently, T2DM, smoking unstable angina prior to STEMI, premature MI and previously reported MI were determined frequently in patients with 786CC genotype than in other individuals. Therefore, all cohorts were comparable in serum concentrations of troponin T, CK-MB, NT-proBNP, total cholesterol and high-density lipoprotein cholesterol. Additionally, serum levels of low-density lipoprotein cholesterol in patients with CC-genotype were significantly higher to those who had other genotypes of eNOs gene.

Table 2 is presented STEMI risk according to TIMI score, STEMI localization and number of damaged coronary arteries in patients included in the study depending on major / minor alleles. There were not significant differences between three cohorts in TIMI risk, STEMI localization, number of damaged coronary arteries, and complication of MI, while damages in left anterior descending artery were found frequently in patients with CC genotype of eNOs gene. On contrary, left main damage was determined frequently in individuals with TT genotypes than in those who had TC genotype, but there was not sufficient difference between rate of left main damage between patients with CC genotype and TC / TT genotypes. Additionally,

Table 3 Hemodynamics in STEMI patients enrolled in the study.

Parameters	TT genotype (n = 73)	TC genotype (n = 64)	CC genotype (n = 40)	P value
HR, per min	80.81 ± 16.68	74.17 ± 14.84	77.00 ± 16.64	PTT-CC = 0.012
SBP, mmHg	138.48 ± 27.13	136.70 ± 29.00	139.00 ± 27.52	NS
DBP, mmHg	82.14 ± 13.18	80.92 ± 12.72	80.00 ± 13.88	NS
LV EDV, ml	137.60 ± 33.36	131.91 ± 45.47	149.72 ± 44.63	NS
LV ESV, ml	65.08 ± 23.34	60.98 ± 31.27	77.32 ± 36.96	PTC-CC = 0.065
LA, cm	4.14 ± 0.60	4.09 ± 0.44	4.22 ± 0.43	NS
LV EF, %	51.25 ± 8.23	54.59 ± 8.96	50.84 ± 11.96	NS
E/A, unit	1.00 ± 0.53	1.03 ± 0.46	1.16 ± 0.52	NS
LV MM, g	266.39 ± 70.88	249.63 ± 74.72	268.98 ± 93.31	NS

DBP: diastolic blood pressure; HR: heart rate; LA: left atrium; LVEDV: left ventricular end diastolic volume; LVESV: left ventricular end systolic volume; LVEF: left ventricular ejection fraction; LVMM: left ventricular myocardial mass; SBP: systolic blood pressure.

there were not significant different between patients' cohorts in complications of MI apart from adverse LV remodeling at 6 month. In fact, total amount of STEMI patients with adverse LV remodeling was identified in 46 individuals. eNOs gene polymorphism was dispensed as following: 19.2% - TT genotype, 26.6% - TC genotype and 37.5% - CC genotype ($p = 0.033$). Hemodynamics in STEMI patients enrolled in the study is reported in Table 3. Apart from HR and LV end-diastolic volume other hemodynamic performances did not distinguished between patients' cohorts.

There were correlations between 786CC polymorphism in eNOs gene and adverse LF remodeling ($r = 0.48$; $p = 0.001$), LDL cholesterol ($r = 0.32$; $p = 0.012$), T2DM ($r = 0.30$; $p = 0.042$), DBP ($r = -0.26$; $p = 0.048$), unstable angina prior to STEMI ($r = 0.25$;

Table 4 The factors contributing late adverse LV remodeling at 6-month after STEMI: The results of univariate and multivariate logistic regression analysis.

Data	Depending variable: late adverse LV remodeling							
	Univariate regressive logistic analysis				Multivariate regressive logistic analysis			
	β -coefficient	OR	95% CI	P	β -coefficient	OR	95% CI	P
786CC genotype of eNOS	1.584	4.873	1.4093 – 16.8481	0.012	1.573	4.823	1.5349 – 15.1552	0.007
Smoking	0.513	1.670	0.3756 – 7.4222	0.501	-	-	-	-
T2DM	0.351	0.704	0.2961 – 1.6748	0.428	-	-	-	-
Previous MI prior to STEMI	0.149	0.862	0.0713 – 4.3338	0.857	-	-	-	-
Stable CAD prior to STEMI	0.440	1.552	0.3988 – 6.0419	0.526	-	-	-	-
Unstable CAD prior to STEMI	0.783	2.318	1.0611 – 4.1522	0.046	0.716	1.232	0.9815 – 4.1772	0.162
Multiple coronary vessel injury	0.224	0.800	0.1766 – 1.2622	0.337	-	-	-	-
LDL cholesterol	0.726	1.427	0.9388 – 3.229	0.663	-	-	-	-

OR: odds ratio; T2DM: type 2 diabetes mellitus; CAD: coronary artery disease; LDL: low-density lipoprotein; LV: left ventricular.

$p = 0.047$) and total quantity of complicated STEMI ($r = 0.23$; $p = 0.042$). Additionally, there were not significant relations between 786CC polymorphism in eNOS gene and multiple coronary vessel injury, STEMI localization, levels of circulating biomarkers of myocardial injury, and amount of damaged coronary arteries.

Using univariate and multivariate regressive logistic analysis (Table 4) we found that 786CC genotype of eNOS gene was independent predictor for late adverse LV remodeling (β -coefficient = 1.57342; odds ration = 4.8231; 95% confidence interval = 1.5349 – 15.1552; $p = 0.0071$).

DISCUSSION

The results of the study have revealed first that the 786CC genotype of eNOS gene was able to independently predict a late adverse cardiac remodeling in STEMI after primary or facilitate PCI. Consequently, we yielded unexpectedly result because other risk factors of poor clinical outcomes, such as T2DM, left main damage, did not corresponded to adverse cardiac remodeling at 6 month after successful PCI. Recent clinical studies have shown a pivotal role of DNA variants in the eNOS gene in vascular nitric oxide production and consequently vascular tone modulating^[25-27]. Yet, SNP in promoter region of eNOS gene was strongly associated with a risk of cardiovascular and renal disease in several populations^[28,29]. Moreover, meta-analysis provided by Kong XZ *et al* (2017)^[30] is reported that 786CC polymorphism in eNOS gene corresponded well with increased risk of primary MI. Therefore, negative impact of the 786CC polymorphism in eNOS gene on a risk of death after MI was determined in manyfore populations^[31-34]. However, previous clinical studies were tackled SNP in eNOS gene with endogenous NO production and vascular endothelial growth factor (VEGF), which is reported as key regulator of angiogenesis, coagulation and tissue repair^[35,36]. We have hypothesized that 786CC polymorphism in eNOS gene suppressing endogenous production of NO and VEGF may worse myocardial perfusion and impair LV function due to persistent ischemia leading to late adverse cardiac remodeling. Although complete opening of large MI-relating artery is discussed as main factor contributing to improve survival in STEMI patients, metabolic regulation of effective myocardial perfusion through small collateral branches could be sufficient trigger to prevent post-MI remodeling and improving survival rate. Indeed, adverse cardiac remodeling strongly associated with short-term as well as long-term mortality regardless of initial TIMI status of STEMI patients after PCI^[37]. In this context, the 786CC polymorphism in eNOS gene could be a cause for restricted effect of endogenous ischemia-induced metabolites (oxidized lipids, free radicals, active molecules of cell adhesion) that regulate vascular tone and prevent small coronary

artery thrombosis^[38]. Taken together, altered production of NO and VEGF in patients with 786CC polymorphism in eNOS gene may potentially contribute to adverse cardiac remodeling that requires to be validated in future investigations. Finally, it can suggest that there is linkage between several polymorphisms affecting not just eNOS gene, but other genes corresponding to VEGF, collagen, matrix metalloproteinases, apoptotic-related and heat-shock proteins, which are involved into post-MI cardiac remodeling and play a protective role from ischemia preventing necrosis. Hens, our results clearly indicate that the 786CC polymorphism in eNOS gene has turn out to be core element of the molecular mechanisms underlying cardiac remodeling that pre-specified a factor for poor clinical outcomes in STEMI patients. We suggest that the risk stratification in the STEMI population based on 786CC polymorphism in eNOS gene identification could be useful for aggressive medical care after PCI and probably pre-treatment procedure implementation.

In conclusion, polymorphism 786CC in eNOS gene was found as an independent predictor for late adverse cardiac remodeling after STEMI.

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AUTHOR CONTRIBUTION

Conception and design: Olga V. Petyunina; writing of the article Olga V Petyunina, Mykola P. Kopytsya, Alexander E. Berezin; critical revision of the article for intellectual content Alexander E Berezin.

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