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Association of Gene Polymorphisms of Some Endothelial Factors with Stent Reendothelization after Elective Coronary Artery Revascularization

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Restenosis remains the main complication after percutaneous coronary interventions in patients with coronary heart disease. The causes of its development include, in particular, genetic factors. We studied polymorphic loci of genes encoding endothelin-1 (*EDN1 rs5370*), endothelin-1 receptor (*EDNRA rs5333*), endothelin-converting enzyme (*ECE1 rs1076669*), and endothelial NO synthase (*eNOS rs1549758*, *eNOS rs1799983*, and *eNOS rs2070244*) in the context of in-stent restenosis development. It was found that the analyzed polymorphisms of the endothelin system genes were more significant for patients aged ≥ 65 years, while the polymorphic loci of the endothelial NO synthase gene (*eNOS rs1799983* and *eNOS rs1549758*) were predominantly associated with time of in-stent restenosis. The obtained results can be useful for comprehensive assessment of the restenosis risk factors and the choice of optimal treatment for patients with coronary heart disease before elective surgical intervention.

Key Words: *percutaneous coronary interventions; restenosis; gene polymorphisms; endothelium*

Percutaneous coronary interventions (PCI) were developed to reduce stenosis or to eliminate occlusion and restore myocardial perfusion using balloon angioplasty and stenting the narrowed artery segment in case of progressive coronary artery atherosclerosis. Interventional surgery is widely used in clinical practice due to its safety and effectiveness in comparison with other modern methods, especially under pathological conditions requiring emergency intervention. However, such interventions damage the endothelium, which leads to delayed in stent endothelization and dysfunction of newly formed endothelial cells and thereby contributes to hyperplasia of the neointima and in-stent restenosis (ISR) in the delayed period [8]. Endothelization requires the balance between activation of smooth muscle cell proliferation and its inhibition [3].

Endothelial dysfunction is one of the key factors contributing to the development of most cardiovascular diseases, including atherosclerosis, hyperlipidemia, hypertension, coronary heart disease (CHD), and chronic heart failure. In recent years, the relationship of endothelial factors and different single nucleotide polymorphisms of their genes with the reendothelization of the stented arterial segments and the rate of reparative process has been studied [4,10,12]. Published data are contradictory, which, on the one hand, can be explained by the heterogeneity of the studied groups of patients, and on the other hand, by insufficient

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knowledge of the endothelial system components. This, in turn, does not allow us to identify the principles of the ISR pathogenesis.

Here we studied the association of polymorphic variants of some genes encoding endothelial factors with coronary artery restenosis occurring after stenting.

MATERIALS AND METHODS

The study enrolled 113 Russian patients of both sexes with CHD (mean age 56.6±10.7 years) who had previously undergone balloon angioplasty with stenting using drug-eluting stents. According to the results of control angiography, they were divided into two groups: patients with ISR (n=54) and patients without ISR (n=59). The patients with ISR were classified into subgroups by the terms of ISR development (<12 months, n=22; >12 months, n=32) and age (under 65 years, n=36; over 65 years, n=18). Exclusion criteria were unstable angina, decompensated heart failure, oncological diseases, renal and hepatic failure.

Genotyping was performed for polymorphic loci of genes encoding endothelin-1 (*EDN1 rs5370*), endothelin-1 receptor (*EDNRA rs5333*), endothelin-converting enzyme (*ECE1 rs1076669*), as well as endothelial NO synthase (*eNOS rs1549758*, *eNOS rs179983*, and *eNOS rs2070744*). Genomic DNA was extracted from the peripheral blood. *EDN1 rs5370* was genotyped using kits for allele-specific PCR (Litech). Gene polymorphisms of the *eNOS* gene were genotyped using the real time PCR method with utilizing commercially available kits (Syntol). To identify polymorphic variants of the *ECE1* and *EDNR*A genes we used restriction fragment length polymorphism-PCR reaction (RFLP-PCR). Amplification conditions for *EDNRA rs5333*: initial melting at 94°C for 5 min followed by 30 cycles (denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec, and elongation at 72°C for 30 sec). Final elongation was performed at 72°C for 10 min. Primers: F: 5'-TTTCTCACTTTCCTTTAGCG-3', R: 5'-ACCTAAGTAATTCACATCGG-3'. Cleavage of amplified fragments was carried out using endonuclease BstAFI (SibEnzyme). The fragment corresponding to allele *T* had a length of 154 bp, the fragments corresponding to allele *C* contained 67 and 87 bp. Amplification conditions for *ECE1 rs1076669*: initial melting at 95°C for 4 min followed by 30 cycles (denaturation at 95°C for 30 sec, annealing at 64°C for 30 sec, and elongation at 72°C for 30 sec). Final elongation was performed at 72°C for 10 min. Primers: F: 5'-TAGAGCCCTGGGCCTGTGAGGAGGAGC-3', R: 5'-CTTACCATCTGTCGGTGGTGTTGATG-3'. Endonuclease BstYI (SibEnzyme) was used to cleave the amplified fragments. The length of fragments corresponding to allele C was 172 and 38 bp, the fragments corresponding to allele *T* contained 111, 61, and 38 bp. The results of restriction digestion were evaluated by vertical PAAG electrophoresis.

The data were analyzed using R-language and SPSS Statistics 20 software (IBM). The χ^2 test and Fisher's exact test were used to compare genotype and allele frequencies between the analyzed groups. The differences were significant at $p \leq 0.05$. The odds ratio (OR) and 95% confidence intervals (95%CI) were also calculated.

RESULTS

The polymorphic locus *ENDRA rs5333* is associated with the development of atherosclerosis and arterial hypertension [9,11], but its significance for the ISR development was not studied. We found that distributions of genotypes in the subgroups of patients with ISR under and above 65 years differed significantly (p=0.025) (Table 1). Minor homozygous *CC* genotype was significantly more frequent in the subgroup of patients with ISR above 65 than in the subgroup of patients under 65 years (OR 4.918; 95%CI 1.328-18.218) and in the group without ISR (p=0.004, OR 14.754; 95%CI 1.866-116.670).

Analysis of the data for the missense variant *ECE1 rs1076669* also showed significant differences between the age subgroups (p=0.003). The incidence of *TT* homozygotes was significantly higher in the subgroup of patients above 65 years than in younger patients (OR 6.343; 95%CI 1.780-22.607) and patients without ISR (p=0.021, OR 3.704; 95%CI 1.295-10.595). As in the case of the above-mentioned polymorphic locus, this SNP was addressed in only few studies [7] and it was not studied in patients with ISR.

EDN1 rs5370 polymorphism was studied at length. Its association with dyslipidemia, CHD, and arterial hypertension has been demonstrated [2]. The analysis of genotype and allele distributions revealed significant differences between the subgroups with earlier (<12 months) and later ISR development (p=0.005) and subgroups of patients with ISR under and above 65 years (p=0.033). It is important to note that *GT* heterozygotes predominated in all the studied subgroups, especially in the subgroup with early ISR. Minor *TT* homozygotes were significantly more frequent than in other subgroups only in the subgroup of patients above 65 years.

The important role of *eNOS rs1799983* and *rs2070744* polymorphisms in atherogenesis, arterial hypertension, angina pectoris, acute myocardial infarction, and hyperhomocysteinemia has been recently demonstrated [5,6]. Some studies showed the association of allele *C* of the *rs2070744* polymorphism with the development of ISR in patients after im-

TABLE 1. Frequencies of Alleles and Genotypes (%) for *EDN1 rs5370*, *EDNRA rs5333*, *ECE1 rs1076669*, *eNOS rs1549758*, *eNOS rs1799983*, and *eNOS rs2070744* Gene Polymorphisms in the Studied Groups

Gene polymorphism	Genotypes and alleles	Group with ISR (*n*=54)	Group without ISR (*n*=59)	Subgroups with ISR			
				under 65 (*n*=36)	over 65 (*n*=18)	<12 months after PCI (*n*=22)	>12 months after PCI (*n*=32)
ENDRA rs5333 (*H323H*)	*TT*	70	75	75	61	64	75
	TC	24	24	22	27	27	22
	CC	6	1	3	12*+	9	3
	T	82	87	86	74.5	77.5	86
	C	18	13	14	25.5	22.5	14
ECE1 rs1076669 (*Thr341Ile*)	*CC*	72	73	75	67	73	72
	CT	20	22	22	16	23	19
	TT	8	5	3	17*+	4	9
	C	82	84	86	75	84.5	81,5
	T	18	16	14	25	15.5	18,5
EDN1 rs5370 (*Lys198Asn*)	*GG*	30	25	33	22	18	38
	GT	65	70	64	67	77°	56
	TT	5	5	3	11*+	5	6
	G	62.5	60	65	55.5	56,5	66
	T	37.5	40	35	44.5	43,5	34
eNOS rs1549758 (*C774T*)	*CC*	30	36	25	39	18	37,5
	CT	28	20	33*	17+	41*°	19
	TT	42	44	42	44	41	43,5
	C	44	46	41.5	47.5	38.5	47
	T	56	54	58.5	52.5	61.5	53
eNOS rs1799983 (*Glu298Asp*)	*GG*	54	53	50	61	45	59
	GT	37	37	39	33	50°	28
	TT	9	10	11	6	5°	13
	G	72.5	71.5	69.5	77.5	70	73
	T	27.5	28.5	30.5	22.5	30	27
eNOS rs2070744 (*T-786C*)	*TT*	39	34	39	39	41	37,5
	TC	39	44	36	44	36	41
	CC	22	22	25	17	23	21,5
	T	58.5	56	57	61	59	58
	C	41.5	44	43	39	41	42

Note. $p<0.05$ in comparison with *group of patients without ISR, +subgroup of patients with ISR under 65 years, °subgroup of patients with ISR later than 12 months after PCI.

plantation of drug-eluting stents [12] and the relationship of heterozygous *GT* genotype of *rs1799983* with ISR [1]. Analysis of the genotype distribution for *eNOS rs1549758* polymorphism revealed a significantly higher frequency of heterozygous carriers in the subgroup of patients with early ISR compared to the subgroup with late ISR (p=0.0005) and the group without ISR (p=0.001, OR 2.78; 95%CI 1.478-5.227). Significant differences were also found between subgroups of patients with ISR under and above 65 years (p=0.016). Heterozygotes were more frequent in the younger age group (OR 2.405; 95%CI 1.233-4.689) and the group without ISR (OR 1.97; 95%CI 1.035-3.749). Analysis of the data on the *eNOS rs1799983* polymorphism revealed no reliable association with ISR in general, but the distributions of genotypes in subgroups with early and late ISR were significantly different (p=0.003). The frequency of minor homozy-

gotes was higher in the subgroup with late ISR, but the frequency of heterozygotes was lower. The results of our study suggest that this polymorphic locus can have a prognostic value in determining possible timing of ISR development in the presence of other predisposing factors. We found no association of the *eNOS rs2070744* polymorphism with ISR.

The results of our study demonstrated the heterogeneity of patients with ISR and the need to stratify them by age and timing of ISR. It was found that the studied polymorphic loci of endothelin system genes were more significant for people over 65 years old. The homozygous minor genotypes for *EDN1 rs5370*, *EDNRA rs5333*, and *ECE1 rs1076669* polymorphisms were significantly more frequent in patients with ISR above 65 years. Polymorphisms of the endothelial NO synthase gene were predominantly associated with the rime to ISR. Heterozygosity for *eNOS rs1799983* and *eNOS rs1549758* predisposed to early ISR, while homozygosity for minor *T* allele for *eNOS rs1799983* was associated with late (>12 months) ISR development after PCI.

REFERENCES

1. Ogorodova LM, Rukin KY, Vintizenko SI, Petrova IV. Association of ENOS gene polymorphisms as a risk factor of coronary in-stent restenosis. Vestn. Ross. Akad. Med. Nauk. 2017;72(2):120-125. doi: 10.15690/vramn796
2. Ahmed M, Rghigh A. Polymorphism in endothelin-1 gene: an overview. Curr. Clin. Pharmacol. 2016;11(3):191-210. doi: 10.2174/1574884711666160701000900
3. Hirschi KK, Ingram DA, Yoder MC. Assessing identity, phenotype, and fate of endothelial progenitor cells. Arterioscler. Thromb. Vasc. Biol. 2008;28(9):1584-1595. doi: 10.1161/ATVBAHA.107.155960
4. Hu H, Jiang C, Li R, Zhao J. Comparison of endothelial cell- and endothelial progenitor cell-derived exosomes in promoting vascular endothelial cell repair. Int. J. Clin. Exp. Pathol. 2019;12(7):2793-2800.
5. Levinsson A, Olin A.C, Björck L, Rosengren A, Nyberg F. Nitric oxide synthase (NOS) single nucleotide polymorphisms are associated with coronary heart disease and hypertension in the INTERGENE study. Nitric Oxide. 2014;39:1-7. doi:10.1016/j.niox.2014.03.164
6. Oliveira-Paula GH, Lacchini R, Tanus-Santos JE. Clinical and pharmacogenetic impact of endothelial nitric oxide synthase polymorphisms on cardiovascular diseases. Nitric Oxide. 2017;63:39-51. doi:10.1016/j.niox.2016.08.004
7. Seremak-Mrozikiewicz A, Barlik M, Perlik M, Kurzawińska G, Drews K. Genetic variability of endothelin-1 system in gestational hypertension and preeclampsia. Ginekol. Pol. 2011;82(5):363-370.
8. Sprague E, Luo J, Palmaz JC. Static and flow conditions: endothelial cell migration onto metal stent surfaces. J. Long Term Eff. Med. Implants. 2017;27(2-4):97-110. doi: 10.1615/JLongTermEffMedImplants.v27.i2-4.10
9. Sugawara J, Tomoto T, Noda N, Matsukura S, Tsukagoshi K, Hayashi K, Hieda M, Maeda S. Effects of endothelin-related gene polymorphisms and aerobic exercise habit on age-related arterial stiffening: a 10-yr longitudinal study. J. Appl. Physiol. (1985). 2018;124(2):312-320. doi: 10.1152/japplphysiol.00697.2017
10. Xu BY, Xiang MX, Wang JA. Endothelial progenitor cells and in-stent restenosis. Curr. Stem Cell Res. Ther. 2015;10(4):364-371. doi: 10.2174/1574888x10666150204150430
11. Yasuda H, Kamide K, Takiuchi S, Matayoshi T, Hanada H, Kada A, Yang J, Miwa Y, Yoshii M, Horio T, Yoshihara F, Nakamura S, Nakahama H, Tei C, Miyata T, Kawano Y. Association of single nucleotide polymorphisms in endothelin family genes with the progression of atherosclerosis in patients with essential hypertension. J. Hum. Hypertens. 2007;21(11):883-892. doi: 10.1038/sj.jhh.1002234
12. Zeng WP, Zhang R, Li R, Luo J.F, Hu XF. Association of the endothelial nitric oxide synthase gene T786C polymorphism with in-stent restenosis in chinese han patients with coronary artery disease treated with drug-eluting stent. PLoS One. 2017;12(1):e0170964. doi:10.1371/journal.pone.0170964