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Analysis of the Role of Carriership of Polymorphic Genotypes of *ESR1*, *eNOS*, and *APOE4* Genes in the Development of Arterial Hypertension in Men

O. V. Dolgikh[1,2], N. V. Zaitseva[1,2], A. E. Nosov[1], A. V. Krivtsov[1], D. G. Dianova[1], O. A. Kazakova[1], E. A. Otavina[1], and I. N. Alikina[1,2]

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We studied the role of the carrier status for polymorphic loci of genes encoding estrogen receptors (*ESR1*), endothelial NO synthase (*eNOS*), and apolipoprotein E (*APOE4*) and products of their expression nitrogen oxide (NO) and apolipoprotein (ApoE) in the development of arterial hypertension in men. Conventionally healthy volunteers and 149 men with clinical manifestations of stage I-II arterial hypertension were examined. In men with arterial hypertension, the frequency of minor allele A of *ESR1* gene was higher (27.5 *vs.* 9.5% in the reference group; χ^2=4.43, p=0.04). The level of NO in the peripheral blood was also higher in the main group (χ^2=3.93, p=0.047). The increase in NO concentration did not depend on the presence of polymorphic genotypes (*GG* and *GT*) of *eNOS* gene, but the decrease in ApoE level in blood serum was associated with *TC* genotype of *APOE4* gene (p=0.04). Our results suggest that minor allele *A* of *ESR1* gene is associated with the development of arterial hypertension in men. Reduced content of ApoE in blood serum of men with arterial hypertension was associated with *APOE4* gene polymorphism. However, increased level of NO did not depend on polymorphic genotypes *GG* and *GT* of *eNOS* gene. These polymorphisms are of specific interest as additional markers of genetic predisposition to the development of arterial hypertension in middle-age men.

Key Words: *arterial hypertension; polymorphism of ESR1, eNOS, and APOE4 genes; nitrogen oxide; apolipoprotein E*

Arterial hypertension (AH) is the most prevalent chronic disease among adult population of developed countries (30-45% of population) and the main factor of development of cardiovascular pathologies, disability, and mortality. AH is characterized by long-term latent period and in most cases is diagnosed at the late stages when damages to the target organs are irreversible. Therefore, the search for early markers of AH is of specific interest for fundamental medicine and clinical practice. AH is a multifactorial polygenic disease determined by risk factors of the patient (nutrition, lifestyle, and bad habits) and environment and genetic predisposition associated with gene polymorphisms and gene associations [3,4].

The spectrum of genes contributing to AH development is broad and includes groups of genes controlling various metabolic and homeostatic systems, which impairments are involved in the pathogenesis of cardiovascular diseases [1,2,7]. Classical understanding of the molecular and genetic mechanisms of AH emphasizes the role of polymorphisms of gene related to the functioning of the rennin—angiotensin system, lipid metabolism, thrombogenesis, and state of the endothelium [6]. Multifactor nature of AH necessitates the search of new candidate genes and their

[1]Federal Scientific Center for Medical and Preventive Health Risk Management Technologies; [2]Perm State National Research University, Perm, Russia. ***Address for correspondence:*** oleg@fcrisk.ru. O. V. Dolgikh

polymorphisms as markers of AH risk. According to published data, polymorphisms of estrogen receptor (*ESR1*), endothelial NO synthase (*eNOS*), and apolipoprotein E (*APOE4*) genes are associated with high risk of cardiovascular pathologies, as their products participate in the regulation of lipid metabolism and vascular tone [6].

Here we studied the role of polymorphic loci of *ESR1*, *eNOS*, and *APOE4* genes and their products (NO, and apolipoprotein E, ApoE) in the development of AH in men.

MATERIALS AND METHODS

We examined 149 men working at the Chemical Plant in the Perm Region. The main group consisted of men with the diagnosis of stage I-II AH (*N*=47; 25-49 years; mean 39.5±7.8 years). The reference group comprised conventionally healthy men without clinical manifestations of AH (*N*=75; 24-47 years, mean 33.2±7.2 years).

Single nucleotide polymorphisms (SNP) of *ESR1*, *eNOS*, and *APOE4* genes associated with high risk of cardiovascular pathologies were studied in all participants (Table 1). Genetic material was isolated from the peripheral blood using DNA-sorb-B kit for DNA extraction from clinical samples (NekstBio). *rs429358* (*APOE*), *rs1799983* (*eNOS*), *rs2228480* (*ESR1*) polymorphisms were genotyped using SNP-screen kits (Syntol) and CFX96 Real Time System device (Bio-Rad). The procedures were performed in the Laboratory of Immunogenetics of the Federal Scientific Center for Medical and Preventive Health Risk Management Technologies. Amplification program: 3 min at 95°C, and 40 cycles of 95°C (15 sec) and 63°C (40 sec). Each series included 4 controls: for normal homozygous genotype, for heterozygous genotype, for variant homozygous genotype, and negative control.

NO level in the peripheral blood was estimated by ELISA using a commercial Total Nitric Oxide and Nitrate/Nitrite Parameter Assay Kit (R&D Systems). ApoE concentration in blood serum was measured by turbidimetry using Standard lipoprotein (a) kit (Human HmbH). Commercial ApoE (Human) was used for construction of the calibration curve.

The results were processed using Statistica 6.0 software. For samples that did not fit normal distribution, the median (Me) and lower and upper quartiles (Q_1, and Q_3) were calculated. The samples were compared by ANOVA using Kruskal—Wallis test and median tests. Allele frequency was calculated from genotypes of the subjects. Genotype distribution in the groups was estimated using Hardy—Weinberg equation and χ^2 test in the codominant and allele inheritance models. Quantitative differences in the allele frequencies were analyzed using odds ratio (OD) test with confidence interval of 95% (95%CI).

RESULTS

The number of men above 40 in the group of patients with AH was significantly higher than in the reference group (χ^2=9.37, p=0.009). The analysis of minor alleles of studied genes showed that the frequency of allele *A* of *ESR1* gene was significantly higher in men with AH (27.5% *vs*. 9.5% in the reference group; χ^2=4.43, p=0.04; Table 2). No significant between-group differences in the frequency of variant genotypes and alleles of *eNOS* gene were observed. However, the frequency of genotypes with minor allele in the homozygote and heterozygote of *APOE4* gene tended to be higher in men with AH (χ^2=4.43, p=0.226; Table 2). It can be concluded that the presence of minor allele *A* in *ESR1* gene (*rs2228480*) is a risk factor for the development of AH in men. This parameter can be also used as an additional marker for estimation of predisposition to AH in middle-aged men. Our findings complement on the data on the interaction between *ESR1* gene polymorphism and risk of cardiovascular diseases [6].

When discussing the role of the *ESR1* gene polymorphism in AH development, we should note that *ESR1* encodes estrogen α-receptor (ERα). SNP in exon 8 of the *ESR1* gene (*rs2228480*) is a substitution of guanine (*G*) with adenine (*A*) in the ligand-dependent functional transcription area, which changes receptor activity [10,12]. It was shown that ERα expression is associated with estrogen-dependent peripheral vasodilatation during ischemia and suppression of atherosclerotic processes in men [11]. It can be hypothesized that enhanced risk of the development of AH and cardio-

TABLE 1. Polymorphic Loci of the Studied Genes Associated with the Risk of Cardiovascular Diseases

Gene	Polymorphic locus	Risk factors associated with AH development	Reference
APOE4	rs429358	Participates in cholesterol transport	[8]
ESR1	rs2228480	ERα ligands control the functions of the endothelium, cardiovascular and nervous systems	[9]
eNOS	rs1799983	Endothelial NO synthase participates in NO synthesis, regulates vascular tone, and thrombogenesis	[11]

TABLE 2. Frequency of Polymorphic Alleles of Genes Associated with the Risk of AH Development

Gene	Allele	Genotype/ Allotype	Main group (*N*=74)		Reference group (*N*=75)		χ^2	*p*	OR (95%CI)
			n	%	*n*	%			
APOE4	rs429358	TT	44	60.0	57	76.0			
		TC	30	40.0	18	24.0			
		CC	0	0	0	0	1.2401	0.2655	
		T		80.0		88.0			
		C		20.0		12.0	1.00	0.18	0.5405 (1.1606-1.8189)
ESR1	rs2228480	GG	41	55.4	65	86.7			
		GA	26	35.1	7	9.3			
		AA	7	9.5	3	4.0	4.7792	0.0917	
		G		73.0		91.3			
		A		27.0		8.7	4.43	0.04	0.2775 (0.0801-0.9611)
eNOS	rs1799983	GG	37	50.0	36	48.0			
		GT	33	44.6	36	48.0			
		TT	4	5.4	3	4.0	0.0283	0.9860	
		G		72.3		72.0			
		T		27.7		28.0	0.01	0.91	1.0545 (0.4020-2.7660)

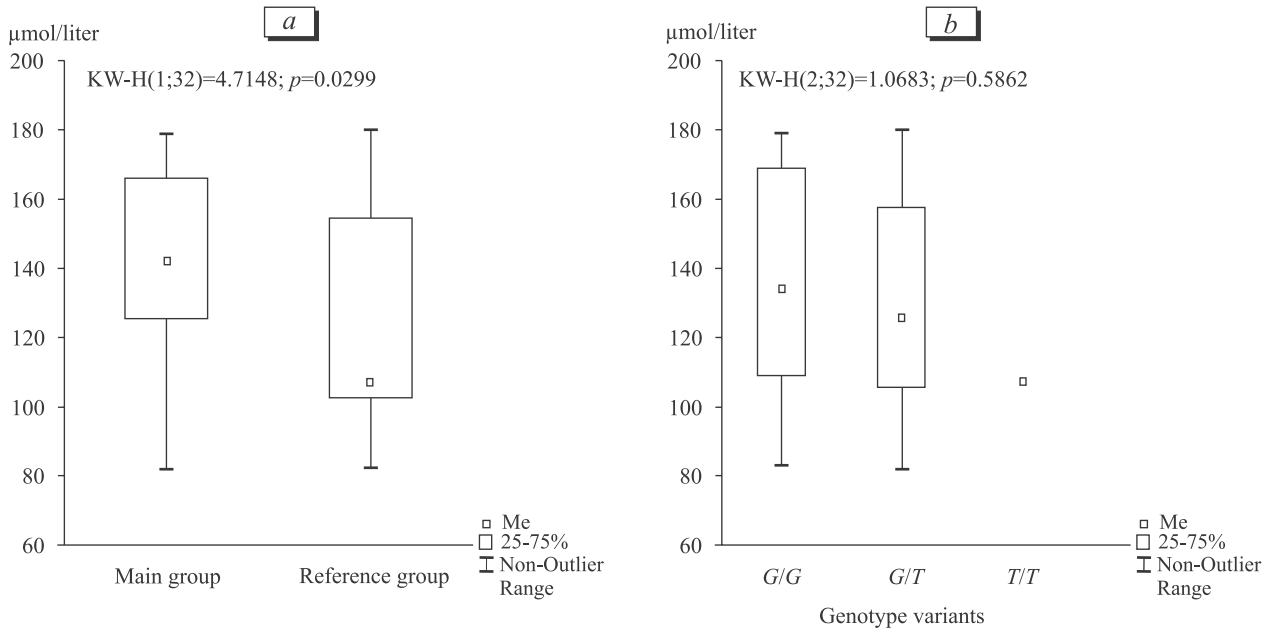

Fig. 1. NO level in blood serum in groups (*a*) and in carriers of homo- and heterozygotes of *eNOS* gene (*rs1799983*) (*b*).

vascular pathologies in men with mutant form of allele *A* in *ESR1* gene (*rs2228480*) are determined by insufficient cardioprotective properties of estrogens [5].

Quantitative analysis of the products of the studied genes in men with AH revealed increased NO level in the peripheral blood (KW-H, p=0.0299; Fig. 1, *a*). This increase did not depend on the presence of polymorphic alleles *GG* and *GT* of *eNOS* gene (Fig. 1, *b*). ApoE level in the blood of examined men with AH tended to decrease, but the difference from the reference group were statistically insignificant (Fig 2, *a*). The reduced ApoE concentration was associated with the presence of minor allele *C* of *APOE4* gene (KW-H, p=0.04; Fig. 2, *b*). ApoE is a plasma protein involved

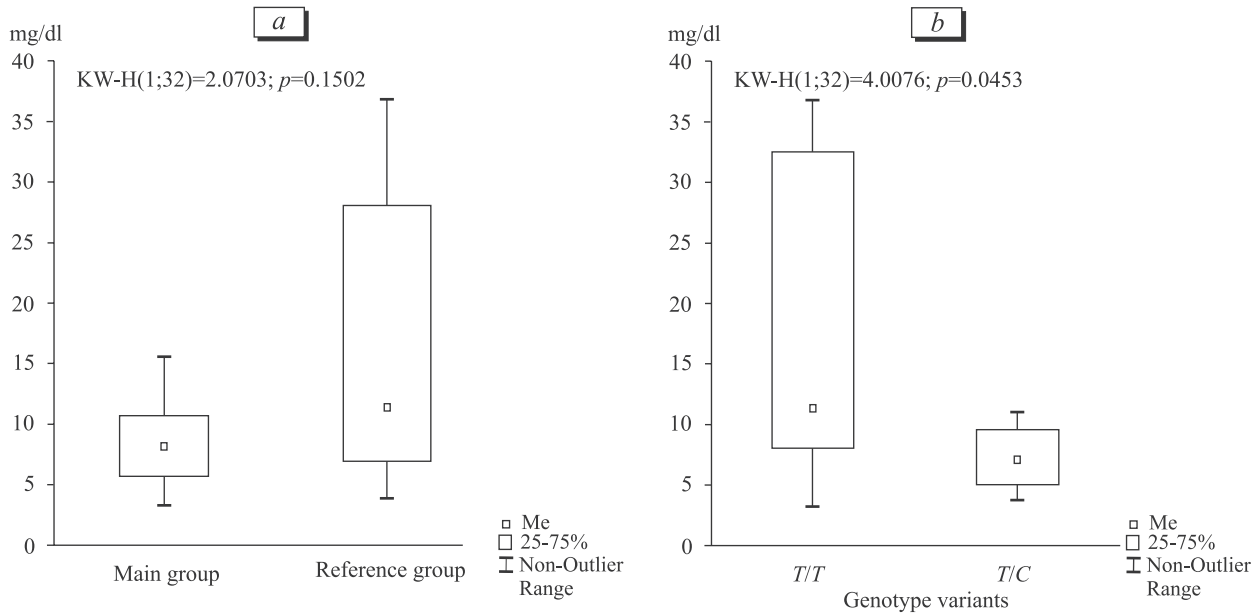

Fig. 2. ApoE level in blood serum in groups (*a*) in carriers of homo- and heterozygotes of *APOE4* gene (*rs429358*) (*b*).

in cholesterol transport [5]. Reduced ApoE concentration promotes cholesterol accumulation in vessels. It can be concluded that the studied polymorphisms of *APOE4* gene are associated with a decrease in ApoE concentration in blood serum and contribute to the development of AH in men. Thus, *APOE4* gene polymorphism plays an important role in AH pathogenesis.

Thus, the revealed peculiarities of genetic polymorphism confirm their significant contribution to AH development in men. The presence of interaction between the polymorphisms of study genes and changed expression of their products is of specific interest for the understanding of existing interactions between genotype and environmental factors.

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