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SINGLE NUCLEOTIDE POLYMORPHISMS OF ADD1 α , AGT, AGTR1 AND AGTR2 GENES IN DIFFERENT ETHNIC GROUPS OF YAKUTIA ARCTIC ZONE RESIDENTS, SUFFERING FROM ARTERIAL HYPERTENSION

DOI 10.25789/YMJ.2018.63.28

ABSTRACT

The article presents the results of the study of the frequencies of occurrence of single nucleotide polymorphisms of the genes *ADD1 α* (1378 G> T), *AGT* (704 T> C and 521 C> T), *AGTR1* (1166 A> C) and *AGTR2* (1675 G> A) groups of residents of the Arctic zone of the RS (Ya), suffering from essential arterial hypertension. Subjects of the research were represented by the most widespread ethnic groups in the territory, including the Slavs, Yakuts, Evens and Evenks. To reveal the above mentioned polymorphisms, a real-time PCR method was used with detection of the melting temperature of duplexes. In the course of the study statistically significant differences between the study groups were identified by the points *ADD1 α* 1378 G> T; *AGT* 521 C> T and *AGTR1* 1166 A> C.

Keywords: arterial hypertension, single nucleotide polymorphisms, *ADD1 α* , *AGT*, *AGTR1*, *AGTR2*, real-time PCR, ethnic groups of the Arctic zone.

Introduction

The Arctic region is unique from many points of view. First, due to geophysical features, the Arctic region is most susceptible to the influence of geomagnetic disturbances, which many authors associate with the development of cardiovascular disorders [1, 2].

This problem is actively studied by the team of authors of this article [10]. In addition, this region has a unique ethnic composition. Now it is known that there are significant differences between people of different ethnic groups, including differences in the course of pathological processes. When studying the pathogenesis of multifactorial diseases, hereditary features are given special attention, because it is the factor that can have significant influence. Primary (essential) hypertension (AH) is a multifactorial disease mainly caused by disturbances in the regulation of arterial blood pressure (BP) at the molecular genetic level. Many researchers attribute these disorders to the presence of point mutations in the genes (single nucleotide substitution) involved in the regulation of blood pressure. If the occurrence of such a mutation exceeds 1% in the studied population, this mutation is called a single nucleotide polymorphism (SNP). In this work, the occurrence of single nucleotide polymorphisms of genes involved in the regulation of BP was studied in various ethnic groups living in the Arctic zone of Yakutia.

Subjects of the research were represented by the most widespread ethnic groups in the territory, including Slavs, Yakuts, Evens and Evenks. Among polymorphisms of genes involved in the regulation of BP, the presence of gene polymorphisms was investigated: *ADD1α* 1378 G> T; *AGT* 704 T> C and 521 C> T; *AGTR1* 1166 A> C; *AGTR2* 1675 G> A.

The role of each of the above polymorphisms in the development of AH and its associated states was examined in the literature. For example, polymorphism of the *ADD1α* gene (1378 G> T), which codes for the α -subunit of the adducin protein, is associated with an increase in sodium reabsorption [6]. In the works of Cusi D. et al. [5] the role of this polymorphism in the development of "salt-sensitive" hypertension was demonstrated. Polymorphisms of the gene encoding angiotensinogen (*AGT* 704 T> C and 521 C> T) are associated with an increase in gene expression and an increase in the plasma level of *AGT* [3, 11, 12]. It is known that angiotensin II interacts with two pharmacologically distinct subtypes of cell surface receptors,

types 1 and 2. Type 1 receptors seem to mediate the cardiovascular effects of angiotensin II [7]. Genotypes *AGTR1* 1166 A> C / C and C/C are associated with a change in the functional activity of the receptor and an increased risk of hypertension [8]. The effects of the receptor for angiotensin II of the second type (*AGTR2*) have not been sufficiently studied to date, but nevertheless, the publications currently available indicate a link between the development of left ventricular myocardial hypertrophy and *AGTR2* polymorphism 1675 G> A [4,9].

Materials and methods of research

A total of 139 volunteers clinically diagnosed with AH participated in this study. Of these, men - n = 42 (30%), women - n = 97 (70%). The average age of the subjects was 50.3 years. The subjects lived in the area of the Arctic zone of Yakutia (Tiksi township). All subjects were divided according to ethnicity into two groups: indigenous (n = 86) and non-indigenous (n = 53) (Table 1).

The group of indigenous inhabitants of the Arctic zone is represented by aboriginal ethnoses who inhabited these territories before the entry of the above-mentioned territories into the Russian state, namely, the Evens, Evenks and Sakha. In turn, as the non-indigenous residents in this study (non-indigenous ethnic groups of the Arctic Zone (NIEGAZ)) comprised of representatives of ethnic groups who migrated to these territories after the annexation, namely Russians, Byelorussians, and Ukrainians who are united in the Slavic ethnoses. Also, the group of non-indigenous included subjects of other ethnic groups, namely Uzbeks, Poles, Chinese, and Kyrgyz.

As the study material, samples of whole venous blood were used, obtained by venipuncture in tubes with EDTA. Reagents of PROBA-RAPID-GENETICS (manufactured by LLC "NPO DNA-Technology", Russia Registration Certificate No. FSS 2010/08695) were used to extract DNA from the samples. Amplification and detection of the polymerase chain reaction products was done on «DT Prime» thermocycler (manufactured by NPO DNA-Technology LLC, Russia Registration Certificate No. FSS 2011/10229), using following reagent kits - A reagent kit for determining genetic polymorphisms associated with risk of development of AH, real-time PCR "CardioGenetics of Hypertension" (manufactured by LLC "NPO DNA-Technology", Russia Registration Certificate No. FF 2010/08414).

The study of all volunteers included

the definition of polymorphisms of seven genes at five points, namely: *ADD1α* 1378 G> T; *AGT* 704 T> C and 521 C> T; *AGTR1* 1166 A> C; *AGTR2* 1675 G> A. Polymorphism was studied in real-time mode, with detection of the melting point of duplexes. The results of the samples which contained the internal control sample, after passing the isolation stage, were considered reliable.

The evaluation of the statistical significance between groups of subjects was carried out using the χ^2 criterion. For descriptive statistics, the software package SPSS Statistics version 13.0 was used. Differences were considered statistically significant at $p < 0.05$.

Results and discussion

First of all, we conducted a comparative analysis of occurrence frequency of polymorphisms associated with AH in Even and Evenk ethnoses. The results of this analysis are set out in Table 2.

As can be seen from Table 2, there were no statistically significant differences between Evenk's and Even's ethnic groups. This allowed us to unite these groups into one - "Indigenous Minorities of the Arctic Zone of the Republic of Sakha (Yakutia)" (IMAZ). The absence of statistically significant differences between these groups, in our opinion, can be explained by the presence of a genetic relationship between them, and probably by mis-self-identification of the subjects.

Next, we analyzed the correspondence of the revealed frequency distribution of the occurrence of genetic polymorphisms associated with AH to the Hardy-Weinberg rule. The results of this analysis are presented in Table 3.

Due to the fact that not all the investigated distributions are described by the Hardy-Weinberg rule, it was decided to use the general model of inheritance for our analysis.

Next, we conducted a comparative analysis between the groups IMAZ and Yakuts, the results of which are presented in Table 4.

As can be seen from Table 4, statistically significant differences between the

Table 1

Ethnic composition of the subjects

	Indigenous ethnic groups of the Arctic zone			Non-indigenous ethnic groups
n	83			56
%	59,71			40,29
	Yakuts	Evens	Evenks	Slavs and oth.
n	35	24	20	56
%	25,18	17,27	14,39	40,29

groups IMAZ and Sakha were found for polymorphisms of the gene ADD1 α (1378 G> T) ($p = 0.01$) and AGT gene (521 C> T) ($p = 0,03$).

Then, we made a comparison between the groups "NIEGAZ" and "IMAZ". The results of this comparison are given in Table 5.

As can be seen from Table 5, the group "IMAZ" statistically significantly differs from the group "NIEGAZ" in two points. The most pronounced differences are observed between the above groups at the points ADD1 α 1378 G> T and AGTR1 1166 A> C.

Next, we compared the "NIEGAZ" group and the Yakuts group. The results of this comparison are presented in Table 6.

Table 6 shows that the groups "NIEGAZ" and "Sakha" statistically significantly differ in two points: ADD1 α 1378 G> T; AGTR1 1166 A> C.

Summarizing the results obtained in the course of the study, it can be established that statistically significant differences between the study groups are available for occurrence frequencies of following polymorphisms: ADD1 α 1378 G> T; AGT 521 C> T and AGTR1 1166 A> C. For these polymorphisms, we calculated the occurrence frequencies of individual alleles, which are presented in Table 7.

As can be seen from Table 7, the unfavorable allele of the T gene ADD1 α (1378 G> T) is most often found in the "IMAZ" group, 1.58 times less frequently, this allele occurs in the Sakha group and 4.04 times less frequently in the NIEGAZ. The unfavorable allele of the T gene of the AGT gene (521 C> T) is most often found in the NIEGAZ and "Yakut groups, the differences between them are minimal, but in the IMAZ group this allele is found 2.92 times less frequently than in the group NIEGAZ. The unfavorable allele of the AGTR1 gene (1166 A> C) is most often found in the "NIEGAZ" group, in comparison with the "IMAZ" group (about 3.39) and the "Sakha" group (about 4.97).

Conclusion

The results obtained in this study indicate that there is a statistically significant difference between the different ethnic

Table 2
Occurrence frequency of genotypes variants of the investigated genes in ethnic groups Evens and Evenks

		Frequency of occurrence		χ^2	p
		Evens (n=28)	Evenks (n=20)		
ADD1 α	G/G	3	4	1,06	0,59
	G/T	19	11		
	T/T	6	5		
AGT 704	T/T	4	2	0,64	0,73
	T/C	15	13		
	C/C	9	5		
AGT 521	C/C	27	17	2,34	0,31
	C/T	1	2		
	T/T	0	1		
AGTR1	A/A	24	18	0,20	0,91
	A/C	4	2		
	C/C	0	0		
AGTR2	G/G	6	6	0,74	0,69
	G/A	13	7		
	A/A	9	7		

groups of people living in the Arctic zone of the Sakha Republic (Yakutia) in distribution of the occurrence frequency of genes polymorphism involved in the regulation of blood pressure, namely: ADD1 α 1378 G> T; AGT 521 C> T and AGTR1 1166 A> C. There were no statistically significant differences in distribution of occurrence frequency of the polymorphisms AGT 704 T> C and AGTR2 1675 G> A. In our opinion, the revealed differences are due to traditionally and historically formed features of the formation of the studied ethnic groups. At the same time, the revealed peculiarities in the distribution of occurrence frequency of

Table 5
Results of a comparative analysis of the occurrence frequency of the investigated genetic polymorphisms in the groups «IMAZ» and «NIEGAZ»

		Frequency of occurrence (N)		χ^2	p
		IMAZ (n=48)	NIEGAZ (n=56)		
ADD1 α	G/G	7	41	39,70	3,0E-9*
	G/T	30	15		
	T/T	11	0		
AGT 704	T/T	6	14	2,65	0,27
	T/C	28	29		
	C/C	14	13		
AGT 521	C/C	44	42	5,03	0,08
	C/T	3	11		
	T/T	1	3		
AGTR1	A/A	42	35	9,07	0,01*
	A/C	6	18		
	C/C	0	3		
AGTR2	G/G	12	23	3,34	0,19
	G/A	20	16		
	A/A	16	17		

Table 3
Results of the analysis of the detected frequency distributions of the observed polymorphisms for compliance with the Hardy-Weinberg rule

	ADD1 α	AGT 704	AGT 521	AGTR1	AGTR2
KMHA3 (n=48)					
χ^2	3,21	1,92	6,47	0,21	1,24
p	0,07	0,17	0,01*	0,64	0,27
Sakha (N=35)					
χ^2	0,01	1,32	0,76	0,07	6,78
p	0,93	0,25	0,38	0,79	0,009*
NIEGAZ (N=56)					
χ^2	1,34	0,07	3,15	0,12	9,97
p	0,25	0,79	0,08	0,73	0,002*

In table 3-6 * $p \leq 0,05$.

Table 4
Results of a comparative analysis of occurrence frequency of the investigated genetic polymorphisms in the groups IMAZ and Yakuts

		Frequency of occurrence (N)		χ^2	p
		IMAZ (n=48)	Yakuts (n=35)		
ADD1 α	G/G	7	15	8,61	0,01*
	G/T	30	16		
	T/T	11	4		
AGT 704	T/T	6	9	3,47	0,18
	T/C	28	14		
	C/C	14	12		
AGT 521	C/C	44	26	6,76	0,03*
	C/T	3	9		
	T/T	1	0		
AGTR1	A/A	42	32	0,32	0,85
	A/C	6	3		
	C/C	0	0		
AGTR2	G/G	12	8	3,13	0,21
	G/A	20	9		
	A/A	16	18		

Table 6
Results of a comparative analysis of occurrence frequency of investigated genetic polymorphisms in the groups NIEGAZ and Yakuts

		Frequency of occurrence (N)		χ^2	p
		NIEGAZ (n=48)	Sakha (n=56)		
ADD1 α	G/G	41	15	11,89	0,003*
	G/T	15	16		
	T/T	0	4		
AGT 704	T/T	14	9	1,60	0,45
	T/C	29	14		
	C/C	13	12		
AGT 521	C/C	42	26	2,24	0,33
	C/T	11	9		
	T/T	3	0		
AGTR1	A/A	35	32	9,51	0,009*
	A/C	18	3		
	C/C	3	0		
AGTR2	G/G	23	8	4,65	0,1
	G/A	16	9		
	A/A	17	18		

polymorphisms of genes involved in the regulation of blood pressure can underlie the clinical features of the course of AH in subjects of different ethnic groups. For example, the high frequency of the polymorphism of the gene encoding the adducin protein (ADD1 α 1378 G> T) in the IMAZ and the Yakuts can cause a high level of "salt-sensitive" AH which requires special considerations in treatment. We couldn't find any information on the prevalence of "salt-sensitive" AH in these ethnic groups. Polymorphism of the angiotensinogen gene (AGT 521 C> T), associated with an elevated plasma level of this protein, was three times less frequent in IMAZ and Yakuts than in the others. As for the polymorphisms of the gene coding the receptor for angiotensin type 1 (AGTR1 1166 A> C), the unfavorable allele C is much more frequent (about 4 times) in representatives of non-indigenous ethnic groups. These features, in our opinion, can underlie the inefficiency of some types of antihypertensive therapy in members of this ethnic group.

The work was partially funded by the Russian Foundation for Basic Research. Project number 18-415-140002 p_a.

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Table 7

Occurrence frequency of individual alleles for polymorphisms ADD1 α 1378 G> T; AGT 521 C> T and AGTR1 1166 A> C in the study groups.

		Frequency of occurrence (N)		
		NIEGAZ (n=56)	Yakuts (n=35)	IMAZ (n=48)
ADD1 α	G	0,866	0,657	0,458
	T	0,134	0,343	0,542
AGT 521	C	0,848	0,871	0,948
	T	0,152	0,129	0,052
AGTR1	A	0,786	0,957	0,938
	C	0,214	0,043	0,063