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CORRELATION OF THE M235T POLYMORPHISM OF THE AGT GENE WITH ARTERIAL HYPERTENSION AND ITS RISK FACTORS IN THE INDIGENOUS PEOPLE OF THE ARCTIC TERRITORY OF YAKUTIA

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A case-control study was conducted for the indigenous population living in the Arctic territory of Yakutia to determine the association of the M235T polymorphism of the *AGT* gene with hypertension and its risk factors. A higher average blood pressure, elevated cholesterol and its fractions, a higher incidence of abdominal obesity in carriers of the mutant GG genotype were found both in the general population and separately for people with hypertension. The study shows the contribution of the G allele of the *AGT* gene to the development of hypertension, lipid disorders and abdominal obesity.

Keywords: *AGT* gene, polymorphism, arterial hypertension, risk factors, indigenous people, Yakutia.

Arterial hypertension is the leading risk factor for disability and premature mortality in the global population. As of 2010, 31.1% of the adult population of the world (1.39 billion people) was suffering from hypertension (30.7% of men and 28.8% of women) [10]. In Russia, according to an ESSE-RF epidemiological study, which was conducted in 12 regions, the prevalence of AH was 50.2% (51.1% in men, 49.7% in women) [2]. It is recognized that hypertension is a polygenic multifactorial disease, the genetic role of which has already been proven. Currently, more than 1.500 genetic polymorphisms associated with blood pressure have been identified, which contribute through various pathogenetic mechanisms [7]. A particularly important role belongs to the genes of the renin-angiotensin system (RAS) responsible for vascular tone. The most relevant polymorphisms of the RAS genes for hypertension are angiotensinogen gene polymorphisms (*AGT*). The results of various researches are ambiguous. Despite numerous studies, the degree and reliability of associations vary, for some loci the data are contradictory.

The **aim** of the study was to research the relationship of angiotensinogen (*AGT*) gene polymorphism with arterial hypertension and its risk factors in the indigenous people of the Arctic territory of Yakutia.

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Materials and research methods. The collection of material for the study was carried out in expeditionary conditions in the Arctic territory of Yakutia, including the places of compact residence of the indigenous peoples (Nizhnekolymsky, Verkhnekolymsky and Tomponsky districts). 348 people of the indigenous nationality were surveyed with the continuous method. The sample consisted of an adult population aged 20 to 70 years (225 women and 123 men). The response was 75%. The average age of respondents was 48.16 ± 0.52 years, 49.71 ± 0.63 in women and 44.98 ± 0.91 in men.

Inclusion criteria: representatives of indigenous people (Evens, Chukchi, Yukaghirs, Yakuts).

Exclusion criteria: representatives of non-indigenous nationalities.

The research program included the following sections: a survey on the questionnaire to assess the objective state; informed consent of the respondent to conduct research; anthropometric examination with hip and waist measurement; blood sampling for biochemical studies from the cubital vein in the morning on an empty stomach with a 12-hour abstinence from food. Blood sampling from the cubital vein for molecular genetic studies was carried out in a tube with EDTA. Genomic DNA was isolated from peripheral blood leukocytes by the method of phenol-chloroform extraction. Allelic variants of the *AGT* gene were tested using a polymerase chain reaction with real-time results (real-time PCR). Genotyping of the polymorphic *AGT* gene was performed with the usage of kits (Lytech R&D LLC, Moscow) on the «Real-time CFX96» amplifier (BioRad, USA) in accordance with the manufacturer's instructions. For quality control, 10% of randomly selected samples were subjected to repeated genotyping.

Biochemical methods of the research included blood lipid profile: total cholesterol (TC), LDL Cholesterol, HDL Cholesterol, TG, glucose test.

When judging the incidence of disorders of the blood lipid profile in a population, we used the Russian recommendations of the V revision of Society of cardiology of Russian Federation (VNOK), 2012, into account the European recommendations, 2011. Hypercholesterolemia (HCE) is the level of total cholesterol (TC) ≥ 5.0 mmol/l, the high LDL Cholesterol level≥3.0 mmol/l, the low HDL Cholesterol level ≤1.0 mmol/l in men; 1.2 mmol/l in women. Hypertriglyceridemia (HTG) is the TG level ≥1.7 mmol/l; a hyperglycemia on an empty stomach (a glucose in a blood plasma on an empty stomach ≥ 6.1 mmol/l) or glucose intolerance (a glucose in a blood plasma in 2 hours after glucose loading within ≥7.8 and ≤11.1 mmol/l).

Blood pressure (BP) was measured twice with an OMRON automatic tonometer (Japan) on the right hand in a sitting position with the calculation of the average BP. Hypertension is present at the 140/90 mmHg (2017 ACC/AHA Guideline).

The abdominal obesity (AO) is exposed to the value of the waist measurement (WM) ≥ 80 cm on women, ≥94 cm on men.

The study was conducted according to the Local Ethics Committee protocol YSC CMP on the respondent's informed consent to the processing of personal data and the study.

Statistical data processing was performed using standard methods of mathematical statistics using the SPSS software package (version 19.0). To define the characteristics, the arithmetic mean

(M) and the characteristic's standard error of the mean (m) were calculated. Intergroup differences were evaluated using analysis of variance or non-parametric criteria. When comparing the frequencies of genotypes, the standard χ2 criterion with the Yates correction was used. The relative risk (OR – odds ratio) of disease development at a certain genotype was calculated using the standard formula OR=a/b x d/c, where a and b is the number of patients with and without the mutant genotype, respectively, and d, c is the number of people in the control group with and without the mutant genotype. OR is indicated with a 95% confidence interval. Differences were considered statistically significant at p <0.05.

Results and discussion. In the total population of the indigenous people of the Arctic territory of Yakutia, the frequency distribution of the AA, AG and GG M235T genotypes of the *AGT* gene was 15.5% (n = 54), 45.1% (n = 157), 39.4% (n = 137), respectively, which corresponds to the Hardy-Weinberg equilibrium (χ2 = 0.18, p = 0.66), A alleles - 38.1% (n = 265), G - 61.9% (n = 431).

When comparing the mean values of lipids and glucose depending on a particular AGT gene genotype, we obtained statistically significant differences in all indicators in carriers of heterozygous AG and mutant homozygous GG genotypes. In GG carriers all values were higher: total cholesterol AG 4.93 ± 0. 06 and GG 5.12 ± 0.06, respectively, p = 0.037; HDL 1.32 ± 0.02 and 1.22 ± 0.02 (p = 0.003); LDL 3.12 ± 0.05 and 3.33 ± 0.05, (p = 0.005); TG 1.06 ± 0.02 and 1.21 ± 0.04 (p = 0.005). Our study confirms the contribution of the G allele to impaired lipid metabolism. On the contrary, their average glucose values are significantly lower compared with AG genotype carriers (4.83 ± 0.11 and 4.35 ± 0.08, respectively, p = 0.001). Significant differences were also obtained when comparing the mean values in individuals with homozygous AA and GG genotypes, namely, in TG values (1.03 ± 0.05 and 1.21 ± 0.04, p = 0.029), glucose (4.88 ± 0.14 and 4.35 ± 0.08, p = 0.001).

At analyzing the frequencies of lipid and carbohydrate disorders in respondents, it was found that all carriers of genotypes showed high numbers of hypercholesterolemia (HCE), especially atherogenic HCE, and GG genotype carriers showed the highest frequency of hypo-alpha-cholesterolemia (Hypo-α-CE). The frequencies of HCE, LDL HCE, Hypo-α-CE in the general population did not have significant differences between genotypes. Thus, in individuals with the AA genotype, the frequency of HCE was 46.3%, AG - 41.4%, GG - 49.6%. More than half of the respondents had the highest frequency of LDL HCE in individuals with AA genotype, 64.8% and GG genotype - 59.8%. The frequency of Hypo-α-CE was 33.3% in the AA genotype individuals, AG - 29.9%, GG - 43.1%. The frequency of HTG in the carriers of homozygous AA and GG genotypes significantly differed (5.5% and 17.5%, respectively, p = 0.033). Also significantly higher was the frequency of HG in heterozygous AG carriers compared with individuals with the mutant GG genotype (8.3% and 2.9%, respectively, p = 0.048).

We also conducted a study of the conjugation of AGT polymorphism with the presence of abdominal obesity. Carriers of the AG and GG genotypes (59.2% and 60.6%) had the largest statistically insignificant frequency of AO versus 46.3% of individuals with the AA genotype, thereby indicating a certain conjugacy of the G allele with the presence of AO.

Considering the high frequency of occurrence of AH in the population (53.3%), for further study, the general population of the indigenous people of the Arctic territory of Yakutia was divided into 2 groups. For the study, 2 groups were formed - "case" and "control": "case" - persons suffering from hypertension (175 people), "control" - people without hypertension (173 people). The average age of hypertensive patients was 53.07 ± 0.49 years, those without AH - 38.88 ± 0.60 years.

This case-control study was included to determine the association of *AGT* gene variants with AH and its risk factors.

The frequency of occurrence of M235T genotypes of the *AGT* gene among groups was compared. None of the data sets (Table 1) showed significant deviation from Hardy-Weinberg equilibrium (χ2 = 0.32, p = 0.24 for "case" and χ2 = 4.84, p = 0.02 for "control") and there was no significant difference in the frequencies of genotypes or alleles between hypertensive and normotonic patients, with the exception of the homozygous AA genotype (χ2 = 5.21, p = 0.001, OR = 0.39, 95% CI = 0.27-0.89) (Table 2).

We used 2 types of genetic models in order to verify the connection of the M235T polymorphism of the *AGT* gene with AH (Table 3). Analyzing the models, we found a link between AH and the mutant homozygous genotype GG and G allele in the recessive model. What is also confirmed by a number of foreign studies, in particular, the effect of the G allele and the GG genotype on the risk of developing essential hypertension [4,6,8,9,15,16]. In the Russian study, which included 514 patients, the association of the G allele with the risk of developing hypertension in men with an odds ratio of 1.95 (p = 0.003) was shown [1]. In contrast, in Colombia, Mongolia, the Caucasus, Lebanon, and India, no reliable association of AG and GG genotypes with AH was found [3,5,11,14,18]. It was assumed that the population is heterogeneous in these countries, polymorphism is associated with differences in populations.

Depending on the genotype, we analyzed the average level of systolic blood pressure (SBP) in hypertensive patients. In individuals with arterial hypertension, the average level of SBP in carriers of AA, AG and GG genotypes was 173.53 ± 3.62, 161.72 ± 1.40 and 159.72 ± 1.92 mmHg, respectively, there were significant differences in individuals with the AA genotype compared with others (p = 0.001). We did not detect any special differences in the mean values of SBP in normotonics.

Comparison of mean concentrations of blood lipids and glucose in individuals with and without hypertension was carried out, depending on whether the *AGT* gene is a member of a particular M235T

Table 1

Frequencies of genotypes and alleles of the M235T polymorphism of the *AGT* gene and correspondence to Hardy-Weinberg equilibrium (HWE)

Genotype	Case	HWE	χ^2	p	Control	HWE	χ2	p	Allele	Frequencies of alleles	
										Case	Control
AA	0.097	0.12	0.32	0.24	0.214	0.17	4.84	0.02	A	0.347	0.416
AC	0.497	0.45			0.405	0.49			C	0.653	0.584
CC	0.406	0.43			0.381	0.34					

Table 2

Frequency distribution of M235T genotypes of the AGT gene among persons with and without AH

Genotype	Frequencies of genotypes		χ2	p	OR	95%CI
	Case	Control				
AA	0.097	0.214	5.21	0.001	0.39	0.27-0.89
AG	0.497	0.405	1.71	нд	1.45	0.83-2.54
GG	0.406	0.381	0.13	нд	1.11	0.63-1.96

genotype (Table 4). For all respondents in the "case" values of lipid metabolism, except for HDL cholesterol, and glucose were higher compared to "control". Significant differences were found in the average concentrations of TG in all representatives, total cholesterol in the homozygous carriers of AA and GG genotypes, LDL cholesterol in carriers of the mutant GG genotype, glucose in individuals with the AA and AG genotype. The average concentrations of total cholesterol, atherogenic cholesterol and TG in hypertensive patients were higher with the GG genotype.

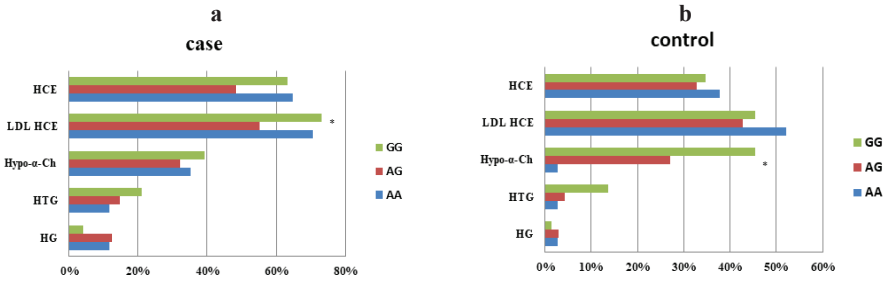
We determined the frequency of lipid and carbohydrate disorders for persons with and without AH, depending on genotype carriership (Fig. 1). In hypertensive patients, all values exceeded those of normotonics. When comparing certain types of lipid disorders and carbohydrate metabolism with respect to one or another genotype, individuals in the "case" and "control" identified significant differences in the frequency of LDL HCE in hypertensive patients of heterozygous and mutant homozygous genotypes, thereby proving the contribution of the G allele in the development of atherogenesis. In normotonics, significant differences were noted in the frequency of Hypo-α-cholesterol, where the highest frequency was observed in GG carriers. In many studies examining the association of the M235T *AGT* gene polymorphism with lipid disorders, no significant reliable links have been identified. Only a few studies confirm the fact that the G allele has a significant effect on increasing the concentration of total cholesterol and atherogenic cholesterol [12].

The study of conjugation of AGT polymorphism in the case and control with the presence of abdominal obesity revealed the highest incidence of AO in individuals with AH - carriers of AG and GG genotypes from 76.1% to 83.9%. In the control, the frequency of AO varied from 28.6% in carriers of the heterozygous genotype to 43.9% in homozygous GG carriers. Both in the group with AH and in the "control", the highest frequency of AO is associated with the G allele, thereby proving its contribution to the development of the metabolic syndrome. This was also confirmed by a number of foreign studies [13, 17].

Table 3

Frequency distribution of genotypes M235T of AGT gene among persons with and without AH according to the dominant and recessive model

Genotype	Frequencies of genotypes		χ2	p	OR	95%CI
	Case	Control				
AA+ AG	0.581	0.618	0.28	0.51	0.86	0.56-1.31
GG	0.418	0.382				
AA	0.095	0.214	9.58	0.001	0.39	0.21-0.72
AG+ GG	0.905	0.786				



1a. The frequency of disorders of lipid and carbohydrate metabolism in individuals with hypertension, depending on the genotypes M235T AGT, 1b. The frequency of disorders of lipid and carbohydrate metabolism in individuals without hypertension, depending on the genotypes M235T AGT. Note: *- p<0,05

Conclusion. Based on our findings, there is, indeed, a single genetic component in the implementation of hypertension and its risk factors for development, such as lipid disorders and abdominal obesity. Proof of this are higher average blood pressure, elevated cholesterol and its fractions, a higher incidence of abdominal obesity in carriers of the mutant GG genotype both in the general population of the indigenous people of the Arctic territory of Yakutia, and separately for people with arterial hypertension. Thus, the genetic mechanisms of hypertension in the group of patients with arterial hypertension are realized through the G allele, which programs obesity, increased pressure, and lipid metabolism disorders.

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Table 4

Mean levels of lipid spectrum and blood glucose in hypertensive patients and those without hypertension depending on the M235T genotypes of the *AGT* gene (M±m)

Blood parameters	AA genotype			AG genotype			GG genotype		
	case	p	control	case	p	control	case	p	control
TC	5.16±0.11	<0.05	4.74±0.16	4.98±0.08	>0.05	4.78±0.10	5.29±0.07	<0.01	4.74±0.09
LDL	3.30±0.08	>0.05	3.06±0.12	3.17±0.06	>0.05	2.98±0.08	3.47±0.06	<0.01	3.04±0.08
HDL	1.32±0.06	>0.05	1.26±0.05	1.29±0.02	<0.05	1.40±0.04	1.22±0.02	>0.05	1.23±0.03
TG	1.17±0.08	<0.02	0.90±0.06	1.14±0.03	<0.01	0.87±0.04	1.29±0.05	<0.05	1.05±0.06
glucose	5.37±0.24	<0.01	4.43±0.14	5.10±0.15	<0.01	4.15±0.10	4.40±0.10	>0.05	4.24±0.10

velopment of new technologies of treatment and risk prediction of hypertension and insult in the Republic of Sakha (Yakutia)" (Government contract No. 1133).

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