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Association of the *AGT* Gene M235T (rs699) Polymorphism with Arterial Hypertension and Metabolic Risk Factors in the Indigenous People of Yakutia

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Abstract

The research objective was to study the association of the *AGT* rs699 missense SNP with arterial hypertension (AH) and metabolic risk factors among indigenous people of the Arctic territory of Yakutia. The obtained data show that representatives of indigenous people of the Arctic territory of Yakutia with the homozygous GG genotype for the *AGT* SNP rs699 are characterized by high levels of systolic blood pressure. The carriage of the GG genotype in AH patients is associated with a high frequency of hypo-HDL cholesterolemia. The carriage of GG genotype in AH patients, compared to subjects without AH, is characterized by higher blood levels of total cholesterol, LDL-C, and triglycerides and is associated with a high frequency of abdominal obesity. Thus, the *AGT* rs699 missense SNP was found to be associated with metabolic risk factors in indigenous AH persons of the Arctic territory of Yakutia. (**International Journal of Biomedicine. 2019;9(4):287-291.**)

Key Words: *AGT* gene • polymorphism • arterial hypertension • risk factors • indigenous people • Yakutia

Abbreviations

AO, abdominal obesity; A**H**, arterial hypertension; **BP**, blood pressure; **FPG**, fasting plasma glucose; **GWAS**, Genome-wide association studies; **HDL-C**, high-density lipoprotein cholesterol; **HP**, hip circumference; **HWE**, Hardy-Weinberg equilibrium; **LDL-C**, low-density lipoprotein cholesterol; **TC**, total cholesterol; **TG**, triglycerides; **RAS**, renin–angiotensin system; **SBP**, systolic blood pressure; **WC**, waist circumference.

Introduction

Arterial hypertension (AH) 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 was suffering from hypertension (30.7% of men and 28.8% of women).[1] 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] AH is considered a multifactorial disease. From the early and negative GWAS for hypertension within the Wellcome Trust Case Control Consortium[3] to the more recent reports,[4-6] 10,280 genetic variants have been associated with risk of high BP. The gene encoding angiotensinogen (AGT) has been implicated in hypertension both through genetic linkage studies and by allelic association. The *AGT* rs699 missense SNP is a T to C substitution in the exon 2, resulting in a functional methionine (M) to threonine (T) substitution at codon 268 (M268T). Previously, rs699 was positioned to the amino acid 235, and the SNP is therefore referred to as M235T. The rs699 threonine variant is associated with higher plasma AGT levels and higher BP.[7] Despite numerous studies, the results are ambiguous and the degree and reliability of associations vary.

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The research objective was to study the association of the *AGT* rs699 missense SNP with AH and metabolic risk factors among indigenous people of the Arctic territory of Yakutia.

Materials and Methods

Material was collected under expeditionary conditions in the Arctic territory of Yakutia, including places of compact residence of the indigenous peoples (Nizhnekolymsky, Verkhnekolymsky and Tomponsky districts). A total of 348 indigenous people of Yakutia were examined. The sample consisted of an adult population aged between 20 and 70 years (225 women and 123 men). All subjects were divided into 2 groups: the Case group consisted of 175 patients with AH; the Control group included 173 people without elevated BP. The average age of hypertensive patients was 53.07±0.49 years, those without AH - 38.88±0.60 years.

The research program included the following sections:

- Anthopometrical reference data: the measurements of BMI (kg/cm^2), WC (cm) and HC (cm)
- Assessment of BP by Korotkov's method. 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
- Assessment of FPG, OGTT, and blood levels of TC, TG, HDL-C, and LDL-C

Glucose and lipid metabolism disorders were diagnosed according to the Russian national recommendations (the All-Russian Scientific Society of Cardiologists [VNOK, 2009])[8] based on the IDF consensus criteria (2006)[9]: TG≥1.7 mmol/l; HDL-C<1.0 mmol/l in males and <1.2 mmol/l in females; LDL-C>3.0 mmol/l; FPG >6.1 mmol/l; IGT 2Hr PG ≥7.8 mmol/l and ≤11 mmol/l. Abdominal obesity (AO) was confirmed at WC ≥ 94 cm in males and ≥ 80 cm in females.

The diagnosis of AH was based on 2017 ACC/AHA Guideline for or the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults.[10]

Genotyping of the AGT rs699 missense SNP was performed in the laboratory of molecular genetics at YSC CMP. From each patient, 2mL of peripheral blood were drawn into an EDTA tube. Genomic DNA was isolated from the peripheral blood leukocytes using standard phenol–chloroform extraction technique. Allelic variants of the AGT rs699 missense SNP were tested by real-time PCR on the «Real-time CFX96» amplifier (BioRad, USA) using Lytech kits (Lytech R&D LLC, Moscow) in accordance with the manufacturer's instructions. The actual SNP for M235T is a T→C substitution; however, the pyrosequencing was done by using the reverse complement strand, which resulted in an allele call of A→G. For quality control, 10% of samples were randomly repeated, with complete congruence.

The study was approved by the Ethics Committee of the Yakut Science Center of Complex Medical Problems. Written informed consent was obtained from each patient.

Statistical analysis was performed using SPSS (version 19.0). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. Means of 2 continuous normally distributed variables were compared by independent samples Student's t test. Mann-Whitney U test was used to compare means of 2 groups of variables not normally distributed. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Deviation from Hardy-Weinberg equilibrium and differences in allele distributions between the two groups were assessed by $\chi 2$- test with 1 degree of freedom (df). A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

In the general population, the frequencies of the AA, AG and GG genotypes of the AGT rs699 SNP were 15.5% (n=54), 45.1% (n=157), and 39.4% (n=137), respectively, which corresponds to the HWE; the frequencies of the A allele and G allele were 38.1% (n=265) and 61.9% (n=431) (Table 1).

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	0.120			0.214	0.173			A	0.346	0.416
AG	0.497	0.452	1.71	0.19	0.405	0.486	4.85	0.03	G	0.654	0.584
GG	0.406	0.428			0.382	0.341					

In the general population, we found significant differences in average values of TC, LDL-C, and TG, depending on the carriage of genotypes of the AGT rs699 SNP. Thus, in carriers of the GG homozygous genotype, all values were higher than in AG heterozygotes and AA homozygotes. On the contrary, the average FPG level was significantly lower in GG carriers than in AG carriers and AA carriers. Carriers of all genotypes showed a high frequency of hypercholesterolemia, hyper-LDL cholesterolemia and hypo-HDL cholesterolemia without significant differences between genotypes. Thus, in the general population, the average frequency of hypercholesterolemia, hyper-LDL cholesterolemia, and hypo-HDL cholesterolemia was 45.8%, 63.3%, and 35.4%, respectively. The frequency of hypertriglyceridemia was as follows: AA carriers - 5.5% and GG carriers - 17.5% (P=0.033). The frequency of an increased FPG level was significantly higher in AG heterozygotes than in individuals with the mutant GG genotype (8.3% and 2.9%, respectively, P=0.048). In the general population, the frequency of AO in GG carriers, AG carriers and AA carriers was as follows: 60.6%, 59.2%, and 46.3%, respectively (P=0.174).

Considering the high frequency of AH in the general population (53.3%), we conducted a case-control study that included 175 AH persons (Case group) and 173 normotensive

persons (Control group) to determine the association of the *AGT* rs699 SNP with hypertension and metabolic risk factors among indigenous people of the Arctic territory of Yakutia.

We did not find statistically significant differences in the frequency distribution of the AG and GG genotypes between the Case group and the Control group. The frequency of the AA genotype was lower in the Case group than in the Control group: 9.7% and 21.4% (*P*=0.009). The distribution of the genotype frequency was not in HWE for the Control group (χ2=4.85, *P*=0.03) (Table 1). The occurrence of the departure from HWE in controls is probably due to population substructure.

We further used the two types of genetic models (Dominant and Recessive models of inheritance) to test the association between the *AGT* rs699 SNP and AH; the results are shown in Tables 2 and 3. We found a link between AH and the mutant homozygous GG genotype and the heterozygous AG genotype in the dominant model (OR=2.53, 95% CI=1.36-4.69, *P*=0.003). A number of studies have also found an association of the G allele and the GG genotype with the risk for developing hypertension.[11-16] The Russian study, which included 514 patients, found an association of the G allele with the risk for developing hypertension in men, with an odds ratio of 1.95 (*P*=0.003).[17] However, a number of studies did not find a reliable association of AG and GG genotypes with AH.[18-22]

Table 2.

Dominant model of inheritance (df = 1)

Genotype	Genotype frequencies		χ^2	*P*	OR	95% CI
	Case (n=175)	Control (n=173)				
AG+GG	0.903	0.786	9.04	0.003	2.53	1.36-4.69
AA	0.097	0.214			0.40	0.21-0.73

Table 3.

Recessive model of inheritance (df = 1)

Genotype	Genotype frequencies		χ^2	*P*	OR	95% CI
	Case (n=175)	Control (n=173)				
GG	0.406	0.382	0.21	0.64	1.11	0.72-1.70
AA+AG	0.594	0.618			0.90	0.59-1.39

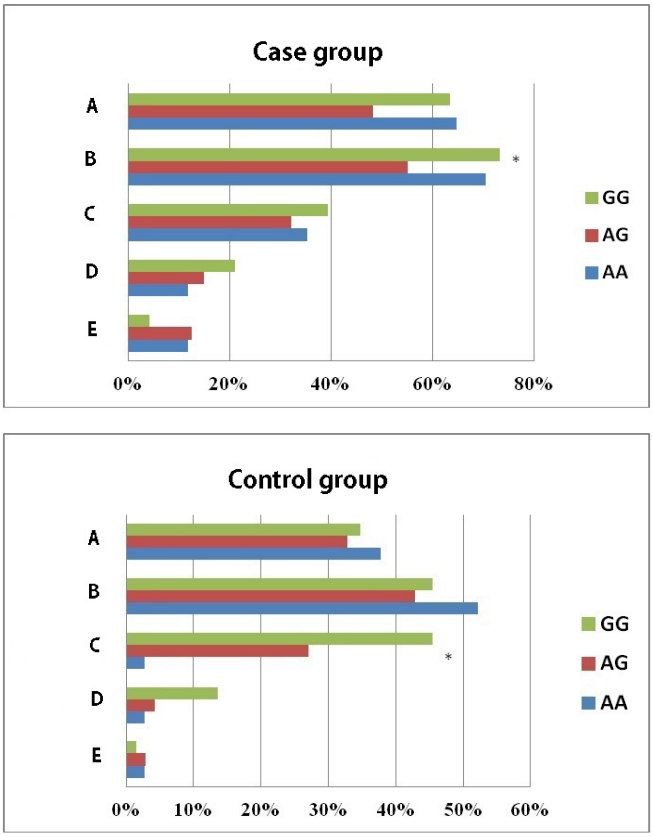

Figure 1. *The frequency of dyslipidemia and carbohydrate metabolism in individuals with and without AH, depending on the carriage of genotypes of the AGT rs699 SNP.*

*A-hypercholesterolemia; B-hyper-LDL cholesterolemia; C-hypo-HDL cholesterolemia; D-hypertriglyceridemia; E-increased FPG level; *- P<0.05*

In Group 1 patients with AH, the average level of SBP in carriers of AA, AG and GG genotypes was 159.72±1.92 mmHg, 161.72±1.40 mmHg and 173.53±3.62 mmHg, respectively (F=6.6763, *P*=0.0016).

Table 4 presents the relationship between *AGT* genotype carriage and parameters of lipid and glucose metabolism in patients with and without AH. The TG level was significantly higher in the Case group regardless of the genotype carriage. In GG carriers, the blood levels of TC and LDL-C were significantly higher in the Case group than in the Control group. The AG carriers in the Case group had significantly lower HDL-C values than in the Control group. In AA and AG carriers, the FPG level was significantly higher in the Case group than in the Control group.

Table 4.

Mean levels of the parameters of lipid spectrum and blood glucose in indigenous people with and without AH depending on carriage of genotypes of the AGT rs699 SNP

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-C	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-C	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
FPG	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

In the Case and Control groups, we found a significantly higher incidence of hypo-HDL cholesterolemia in GG carriers than in carriers of the AA and AG genotypes (Fig. 1). It should be noted that only a few studies confirm the association of the G allele with the presence of hypercholesterolemia.[23]

In the Case group, the highest incidence of AO was found in carriers of the AG and GG genotypes (76.1% and 83.9%). In the Control group, the frequency of AO varied from 28.6% in AG carriers to 43.9% in GG carriers. It should be noted that a contribution of the GG genotype to the development of metabolic syndrome was confirmed by a number of studies.[24,25]

Conclusion

The obtained data show that representatives of indigenous people of the Arctic territory of Yakutia with the homozygous GG genotype for the *AGT* SNP rs699 are characterized by high levels of SBD. The carriage of the GG genotype in AH patients is associated with a high frequency of hypo-HDL cholesterolemia. The carriage of GG genotype in AH patients, compared to subjects without AH, is characterized by higher blood levels of TC, LDL-C, and TG and is associated with a high frequency of AO. Thus, the *AGT* rs699 missense SNP was found to be associated with metabolic risk factors in indigenous AH persons of the Arctic territory of Yakutia.

Competing Interests

The authors declare that they have no competing interests.

Sources of Funding

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