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Association of *AGT* (T704C) and *NOS3* (G894T) Gene Polymorphisms with Treatment-Resistant Hypertension in the Uzbek Population

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Abstract

The aim of our study was to assess the effect of polymorphic markers of the *AGT* T704C (M235T) rs699 and *NOS3* G894T **(**Glu298Asp) rs1799983 SNPs on the risk of the development of treatment-resistant hypertension (TRH).

Methods and Results: The study included 178 patients (mean age of 56.67±11.12 years) with AH Grades 1-3 (ESC/ESH, 2018), who were on outpatient treatment at the Republican Specialized Scientific and Practical Medical Center for Cardiology. The effectiveness of therapy was assessed by achieving the target BP level according to 2018 ESH/ESH Guidelines for the management of AH. The primary target level for SBP and DBP was <140 mmHg and <90 mmHg, respectively.

Genomic DNA samples were isolated from the peripheral blood leukocytes by using the Diatom™ DNA Prep 200 Kit (Isogen Laboratory LLC, Moscow) according to manufacturer`s protocol. A multiplex RT-PCR assay was used to detect the *AGT* T704C (M235T) rs699 SNP and *NOS3* G894T (Glu298Asp) rs1799983 SNP.

We studied the distribution of the *AGT* T704C (M235T) rs699 SNP in 61 Uzbek patients with TRH (cases) and 117 Uzbek patients with non-TRH (controls) (Group 1) and the distribution of the *NOS3* G894T **(**Glu298Asp) rs1799983 SNP in 61 Uzbek patients with TRH (cases) and 115 Uzbek patients with non-TRH (controls) (Group 2).

Our results indicate a significantly greater accumulation of the C allele and CC genotype of the *AGT* T704C (M235T) rs699 SNP among TRH patients than among patients with non-TRH. We found a significant association between the *AGT* T704C (M235T) rs699 SNP and the risk of TRH under the multiplicative genetic model (C vs. T: OR=1.85, 95% CI: 1.17-2.92, *P*=0.008), additive model (CC vs.TT vs. TC; OR=3.00, 95% CI: 1.56-5.75, *P*=0.009), and recessive model (CC vs. TC+TT; OR=3.00, 95% CI: 1.56-5.75, *P*=0.0008). For the *NOS3* G894T **(**Glu298Asp) rs1799983 SNP, the multiplicative model showed a significant risk of TRH with the carriage of the T allele (OR=1.99, 95% CI: 1.20-3.28, *P*=0.007), and the additive model showed a significant risk of TRH with the carriage of the heterozygous GT genotype (OR=2.25, 95% CI: 1.17-4.33, *P*=0.01). At the same time, the carriage of the G allele (OR=0.5, 95% CI: 0.30-0.83, *P*=0.007) and GG genotype (OR=0.40, 95% CI: 0.21-0.76, *P*=0.01) may be protective against the development of TRH.

Conclusion: Further genetic studies of TRH may help achieve better individual outcomes by optimizing drug therapy based on genetic variation.**(International Journal of Biomedicine. 2023;13(2):210-216.)**

Keywords: treatment-resistant hypertension • angiotensinogen • nitric oxide synthase • single nucleotide polymorphism

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Abbreviations

AH, arterial hypertension; **AGT**, angiotensinogen; **BP**, blood pressure; **BMI**, body mass index; **DBP**, diastolic BP; **CIMT**, carotid intima-media thickness; **HWE**, Hardy–Weinberg equilibrium; **LVH**, left ventricular hypertrophy; **LVMI**, left ventricular mass index; **NOS**, nitric oxide synthase; **SBP**, systolic BP; **SNP**, single nucleotide polymorphism; **TRH**, treatment-resistant hypertension.

Introduction

Treatment-resistant hypertension (TRH) is defined as uncontrolled hypertension on ≥3 antihypertensive medication classes or requiring ≥4 antihypertensive medications to reach their BP goals.[1,2] Among US adults taking antihypertensive medication, the prevalence of apparent TRH was 17.7% (9.2 million persons) when applying the definition in the 2008 Scientific Statement, whereas it was 19.7% (10.3 million persons) using the 2018 Scientific Statement definition.[3] The etiology of TRH appears to be multifactorial. Risk factors for TRH include older age, obesity, impaired renal function, diabetes mellitus, African American race, and other factors, including genetic ones.[4-8]

The question of whether there are specific genetic risk factors for TRH is of great interest, especially considering race and ethnicity. Although previous studies have identified numerous genetic variants associated with hypertension and blood pressure,[9-12] there is little evidence regarding the molecular genetic factors of TRH. To date, the available evidence surrounding pharmacogenomics in TRH is limited and primarily focused on candidate genes.[13-15] In recent years, several studies with an integrated genetic approach, genome-wide association studies (GWASs), have identified some significant susceptibility loci for TRH in the US population.[16-19]

A published paper entitled "Genetic and adverse health outcome associations with TRH in GenHAT" by Lynch et al.[20] evaluated the association between 78 candidate gene polymorphisms and TRH. The main finding was the association of two genetic variants in the *AGT* gene, the M allele of rs699 and the G allele of rs5051, and TRH in white but not in African American subjects.

The M235T molecular variant (T704C, rs699) of the *AGT* gene, encoding a threonine instead of a methionine at residue 235 of the mature protein, has been associated with a higher plasma AGT level and higher BP in patients homozygous for the T allele and occurs among various ethnic populations.[21-13] In a meta-analysis, the TT genotype was associated with a 32% increase in the risk of hypertension in white people but not in non-white people, when compared with the MM genotype.[24]

The most examined rs1799983 polymorphism (also known as G894T or Glu298Asp) is located in exon 7 of the *NOS3* gene and formed by a transversion from guanine (G) to thymine (T), resulting in the replacement of glutamic acid (Glu) residue with aspartic acid (Asp) residue in the NOS3 polypeptide.[25] This genetic mutation reduces the production of NO and subsequently affects the development of AH.[26] In some studies, the T allele of the rs1799983 polymorphism was reported to be associated with a decreased level of NO.[27-29]

Unfortunately, the literature data on genetic studies of resistant arterial hypertension, especially in the Asian population, are limited. A better understanding of genetic risk may improve clinical care for TRH and prevent associated cardiovascular disease morbidity and mortality.

The aim of our study was to assess the effect of polymorphic markers of the *AGT* T704C (M235T) rs699 and *NOS3* G894T (Glu298Asp) rs1799983 SNPs on the risk of TRH development.

Materials and Methods

The study included 178 patients (mean age of 56.67±11.12 years) with AH Grades 1-3 (ESC/ESH, 2018), who were on outpatient treatment at the Republican Specialized Scientific and Practical Medical Center for Cardiology. The effectiveness of therapy was assessed by achieving the target BP level according to 2018 ESH/ESH Guidelines for the management of AH. The primary target level for SBP and DBP was <140 mmHg and <90 mmHg, respectively.

Exclusion criteria were symptomatic hypertension, valvular heart disease, acute coronary syndrome, chronic heart failure (NYHA FC>III), cardiac arrhythmia, history of stroke and myocardial infarction, diabetes, occlusive peripheral arterial disease, renal impairment, severe co-morbidities, orthostatic hypotension.

All patients underwent the following examinations: assessment of traditional risk factors, physical examination, clinical and biochemical laboratory methods, 12-lead ECG, and echocardiography. Office BP was measured using a mercury sphygmomanometer, according to Korotkov's method. BP was measured 3 times, and the means of these measurements were used in the analyses. Echocardiography was carried out according to the recommendations of the American Society of Echocardiography in M- and B-modes using Philips EnVisor C Ultrasound Machine (the Netherlands). LVM was calculated using the formula R. Devereux (1994). Left ventricular hypertrophy (LVH) was defined as LVMI of >95 g/m^2 (for women) and >115 g/m^2 (for men).[30] Carotid intima-media thickness (CIMT) was assessed for both left and right carotid arteries using a 7.5 MHz linear array transducer (Sonoline Versa Pro ultrasound system, Siemens, Germany).

Blood levels of TC, TG, HDL-C, LDL-C, and VLDL-C were determined in the venous blood using automatic biochemical analyzer Daytona (RANDOX, United Kingdom) and RANDOX test systems by the enzymatic colorimetric method. The content of LDL-C was calculated according to Fridvald's formula.

Genomic DNA samples were isolated from the peripheral blood leukocytes by using the Diatom™ DNA Prep 200 Kit (Isogen Laboratory LLC, Moscow, Russia) according to manufacturer`s protocol. The quantity and quality of DNA were determined on a NanoDrop 2000 spectrophotometer (Thermo Scientific™ Wilmington, DE, USA). A multiplex RT-PCR assay was used to detect the *AGT* T704C (M235T) rs699 and *NOS3* G894T (Glu298Asp) rs1799983 SNPs.

Statistical analysis was performed using the statistical software «Statistica» (v10.0, StatSoft, USA). For descriptive analysis, results are presented as mean±standard deviation (SD). Means of 2 continuous normally distributed variables were compared by independent samples Student's t test. The Mann-Whitney U Test was used to compare the differences between the two independent groups (for nonparametric data). Group comparisons with respect to categorical variables were performed using chi-square test. Genetic markers for HWE were tested. Differences in the allele and genotype distribution between the groups were assessed by $\chi 2$-test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated.

Four genetic models were analyzed: the dominant model, the recessive model, the multiplicative model, and the additive model (the Cochran-Armitage trend test). A probability value of $P<0.05$ was considered statistically significant.

The study protocol was reviewed and approved by the Ethics Committee of the Republican Specialized Centre of Cardiology. All participants provided the written informed consent.

Results and Discussion

We studied the distribution of the *AGT* T704C (M235T) rs699 polymorphism in 61 Uzbek patients with TRH (cases) and 117 Uzbek patients with non-TRH (controls) (Group 1). We also studied the distribution of the *NOS3* G894T **(**Glu298Asp) rs1799983 polymorphism in 61 Uzbek patients with TRH (cases) and 115 Uzbek patients with non-TRH (controls) (Group 2). The clinical characteristics of AH patients are presented in Table 1.

In Group 1, the mean age of the 178 AH patients was 56.67±11.12 years, the mean duration of AH was 9.42±5.49 years, and the average SBP and DBP were 166.53±17.68 mmHg and 98.55±12.02 mmHg, respectively. Obesity and overweight were found in 52.8% and 32.0% of cases, respectively. About 87.6% and 74.7% of patients were diagnosed with LVH and increased CIMT, respectively. Dyslipidemia was detected in 77.0% of patients. In Group 2, the mean age of the 176 AH patients was 56.71±11.14 years, the mean duration of AH was 9.56±5.83 years, and the average SBP and DBP were 166.55±17.79 mmHg and 98.89±12.17 mmHg, respectively. Obesity and overweight were found in 52.8% and 31.8% of cases, respectively. About 88.1% and 75.0% of patients were diagnosed with LVH and increased CIMT, respectively. Dyslipidemia was detected in 77.3% of patients. Thus, given the previous data, our AH patients had a high and high-to-very-high cardiovascular risk. In both groups, TRH patients were older than non-TRH patients and had a longer course of AH, higher SBP and DBP, and LVH frequency.

Results of the genotyping of the *AGT* T704C (M235T) rs699 and *NOS3* G894T (Glu298Asp) rs1799983 SNPs are presented in Table 2.

The distribution of polymorphic markers of the *AGT* T704C (M235T) rs699 SNP in TRH patients and non-TRH patients was in HWE. In TRH patients and non-TRH patients, the genotype distribution was as follows: CC=50.8%, CT=32.8%, TT=16.4% and CC=25.6%, CT=53.8%, TT=20.5%, respectively. An analysis of the frequency distribution of alleles of the *AGT* T704C (M235T) rs699 SNP showed that the carriage of the C allele was dominant in TRH patients (67.2% vs. 32.8% for the T allele; P=0.000), compared to non-TRH patients (52.6% vs. 47.4% for the T allele; $P>0.05$).

Analysis of the multiplicative model for the *AGT* T704C (M235T) rs699 SNP showed a significant risk of TRH with the carriage of the C allele (OR=1.85, 95% CI: 1.17-2.92, P=0.008). The additive and recessive models for the *AGT* T704C (M235T) rs699 SNP showed a significant risk of TRH with the carriage of the homozygous CC genotype (OR=3.00, 95% CI: 1.56-5.75, P=0.009) (Table 3).

Table 1.

Clinical characteristics of AH patients in the study groups.

Variable	Group 1 *AGT* T704C (M235T) rs699 SNP				Group 2 *NOS3* G894T (Glu298Asp) rs1799983 SNP			
	Total (n=178)	TRH (cases) n=61	non-TRH (controls) n=117	*P*	Total n=176	TRH (cases) n=61	non-TRH (controls) n=115	*P*
Age, years	56.67±11.12	61.52±9.43	54.06±11.17	0.000	56.71±11.14	61.52±9.43	54.09±11.16	0.000
AH duration, years	9.42±5.49	10.60±5.69	8.77±5.32	0.035	9.56±5.83	10.60±5.69	8.69±5.84	0.039
SBP, mmHg	166.53±17.68	172.42±20.62	162.73±16.31	0.001	166.55±17.79	172.42±20.62	162.91±16.17	0.001
DBP, mmHg	98.55±12.02	101.13±9.60	97.17±13.02	0.038	98.89±12.17	101.13±9.60	97.75±11.23	0.048
BPmean, mmHg	120.92±12.18	124.89±12.35	118.78±11.65	0.001	121.67±13.85	124.89±12.35	119.46±12.70	0.007
BMI, kg/m^2	32.17±5.68	33.53±5.92	31.79±5.61	0.056	32.21±5.72	33.53±5.92	31.86±5.63	0.068
BMI>30 (kg/m^2), %	94 (52.8%)	36 (59.0%)	58 (49.6%)	0.234	93 (52.8%)	36 (59.0%)	57 (49.6%)	0.236
BMI>25<30 (kg/m^2), %	57 (32.0%)	21 (34.4%)	36 (30.8%)	0.626	56 (31.8%)	21 (34.4%)	35 (30.4%)	0.589
LVH, %	156 (87.6%)	59 (96.7%)	97 (82.9%)	0.008	155 (88.1%)	59 (96.7%)	96 (83.5%)	0.01
CIMT ≥0.9 mm, %	133 (74.7%)	48 (78.7%)	85 (72.6%)	0.376	132 (75.0%)	48 (78.7%)	84 (73.0%)	0.407
Dyslipidemia, %	137 (77.0%)	51 (83.6%)	86 (73.5%)	0.130	136 (77.3%)	51 (83.6%)	85 (73.9%)	0.145

P - between cases and controls in Groups 1 and 2.

Table 2.

The distribution of polymorphic markers of the AGT* T704C (M235T) rs699 SNP and *NOS3* G894T *(Glu298Asp)* rs1799983 SNP *in TRH patients and non-TRH patients (controls).

Gene	SNP	Genotype	TRH	HWE	χ^2	*P*	Control	HWE	χ^2	*P*	Allele	Frequency of alleles	
												TRH	Control
AGT	rs699 T704C	TT	0.164	0.107	1.72	0.19	0.205	0.225	0.35	0.56	T	0.328	0.474
		CT	0.328	0.441			0.538	0.499			C	0.672	0.526
		CC	0.508	0.452			0.256	0.276					
NOS3	rs1799983 G894T	GG	0.459	0.463	0.00	1	0.678	0.654	1.42	0.23	G	0.680	0.809
		GT	0.443	0.435			0.261	0.309			T	0.320	0.191
		TT	0.098	0.102			0.061	0.037					

Table 3.

Genetic predisposition to TRH.

Genetic model	Allele, Genotype	Cases	Controls	χ^2	*P*	OR (95%CI)	
		n=61	n=117			OR	95%CI
AGT T704C (M235T) rs699 SNP							
Multiplicative model (χ^2 test, df=1)	T	0.328	0.474	7.05	0.008	0.54	0.34-0.85
	C	0.672	0.526			1.85	1.17-2.92
Additive model ([CATT], xi=[0,1,2], df=1)	TT	0.164	0.205	6.74	0.009	0.76	0.34-1.71
	TC	0.328	0.538			0.42	0.22-0.80
	CC	0.508	0.256			3.00	1.56-5.75
Dominant model (χ^2 test, df=1)	TT	0.164	0.205	0.44	0.51	0.76	0.34-1.71
	TC + CC	0.836	0.795			1.32	0.58-2.97
Recessive model (χ^2 test, df=1)	TT + TC	0.492	0.744	11.28	0.0008	0.33	0.17-0.64
	CC	0.508	0.256			3.00	1.56-5.75
NOS3 G894T (Glu298Asp) rs1799983 SNP							
Genetic model	Allele, Genotype	Cases	Controls	χ^2	*P*	OR (95%CI)	
		n=61	n=115			OR	95%CI
Multiplicative model (χ^2 test, df=1)	G	0.680	0.809	7.29	0.007	0.50	0.30-0.83
	T	0.320	0.191			1.99	1.20-3.28
Additive model ([CATT], xi=[0,1,2], df=1)	GG	0.459	0.678	6.62	0.01	0.40	0.21-0.76
	GT	0.443	0.261			2.25	1.17-4.33
	TT	0.098	0.061			1.68	0.54-5.25
Dominant model (χ^2 test, df=1)	GG	0.459	0.678	8.00	0.005	0.40	0.21-0.76
	GT + TT	0.541	0.322			2.48	1.31-4.70
Recessive model (χ^2 test, df=1)	GG + GT	0.902	0.939	0.82	0.37	0.59	0.19-1.85
	TT	0.098	0.061			1.68	0.54-5.25

The distribution of polymorphic markers of the *NOS3* G894T **(**Glu298Asp) rs1799983 SNP in TRH patients and non-TRH patients were in HWE. In TRH patients and non-TRH patients, the genotype distribution was as follows: GG=45.9%, GT=44.3%, TT=9.8% and GG=67.8%, GT=26.1%, TT=6.1%, respectively, thus GG genotype prevailed in non-TRH patients, compared to TRH patients (χ2=8.005, *P*=0.018). An analysis of the frequency distribution of alleles of the *NOS3* G894T **(**Glu298Asp) rs1799983 SNP showed that the carriage of the G allele was dominant in TRH

patients (68.0% vs. 32.0% for the T allele) and non-TRH patients (80.9% vs. 19.1% for the T allele) with the highest degree of dominance in non-TRH patients vs. TRH patients ($\chi 2$=7.29, *P*=0.007).

Analysis of the multiplicative model for the *NOS3* G894T (Glu298Asp) rs1799983 SNP showed a significant risk of TRH with the carriage of the T allele (OR=1.99, 95% CI: 1.20-3.28, *P*=0.007). Analysis of the additive model for the *NOS3* G894T (Glu298Asp) rs1799983 SNP showed a significant risk of TRH with the carriage of the heterozygous GT genotype (OR=2.25, 95% CI: 1.17-4.33, *P*=0.01). At the same time, the carriage of the G allele (OR=0.5, 95% CI: 0.30-0.83, *P*=0.007) and GG genotype (OR=0.40, 95% CI: 0.21-0.76, *P*=0.01) may be protective against the development of TRH.

It should be noted that data on the study of molecular genetic markers of resistant hypertension are limited, especially in the Asian population. To identify novel genetic loci associated with resistant hypertension in the Japanese population, Takahashi et al.[32] conducted a genome-wide association study with 2705 resistant hypertension cases and 21,296 mild hypertension controls, all from BioBank Japan. The authors identified one novel susceptibility candidate locus, rs1442386 on chromosome 18p11.3 (DLGAP1), achieving genome-wide significance (OR=0.85, 95% CI: 0.81-0.90, $P=3.75\times10^{-8}$), and 18 loci showing suggestive association, including rs62525059 of 8q24.3 (CYP11B2) and rs3774427 of 3p21.1 (CACNA1D).[32] Yugar-Toledo et al.[33] examined 70 resistant, 80 well-controlled hypertensive patients, and 70 normotensive controls. All subjects were genotyped for *ACE* insertion/deletion (rs1799752), *AGT* M235T (rs699), and *NOS3* Glu298Asp (rs 1799983), and the multifactor dimensionality reduction analyses showed that carriers of the *AGT* 235T allele were at increased risk for resistant hypertension, especially if they were older than 50 years.

The vasodilator effect of NO that eNOS produces is very important for maintaining the vascular function,[31] and the G894T polymorphism, which is associated with reduced eNOS expression and activity, and subsequently reduced NO production, could be a potential candidate marker for hypertension development.[34,35] In a study by Shi et al.,[36] a total of 60 eligible articles involving 14,185 cases and 13,407 controls were finally selected. The authors found a significant association between eNOS rs1799983 polymorphism and hypertension under any genetic model (T vs G: OR=1.44, 95% CI 1.26–1.63; GT vs GG: OR=1.34, 95% CI 1.18–1.52; TT vs GG: OR=1.80, 95% CI 1.41–2.31; GT+TT vs GG: OR=1.42, 95% CI 1.25–1.63; TT vs GG+GT: OR=1.68, 95% CI 1.35–2.08; GT vs GG+TT: OR=1.24, 95% CI 1.11–1.40). Jáchymová et al.[37] showed that the T allele of the *NOS* G894T (Glu298Asp) rs1799983 SNP may be a factor in the resistance to conventional antihypertensive therapy.

Despite known advances in genetic research technology, TRH has not yet fully taken advantage of more complex genetic approaches, such as GWAS, genome sequencing, and others used in pharmacogenomics research. Gaining a complete understanding of the genetic background of TRH is critical to predicting individual TRH risk and improving individual outcomes by optimizing drug therapy based on clinical features and genetic risk factors.

Our results indicate a significantly greater accumulation of the C allele and CC genotype of the *AGT* T704C (M235T) rs699 SNP among TRH patients than among patients with non-TRH. We found a significant association between the *AGT* T704C (M235T) rs699 SNP and the risk of TRH under the multiplicative genetic model (C vs. T: OR=1.85, 95% CI: 1.17-2.92, *P*=0.008), additive model (CC vs.TT vs. TC; OR=3.00, 95% CI: 1.56-5.75, *P*=0.009), and recessive model (CC vs. TC+TT; OR=3.00, 95% CI: 1.56-5.75, *P*=0.0008). For the *NOS3* G894T (Glu298Asp) rs1799983 SNP, the multiplicative model showed a significant risk of TRH with the carriage of the T allele (OR=1.99, 95% CI: 1.20-3.28, *P*=0.007), and the additive model showed a significant risk of TRH with the carriage of the heterozygous GT genotype (OR=2.25, 95% CI: 1.17-4.33, *P*=0.01). At the same time, the carriage of the G allele (OR=0.5, 95% CI: 0.30-0.83, *P*=0.007) and GG genotype (OR=0.40, 95% CI: 0.21-0.76, *P*=0.01) may be protective against the development of TRH. Further genetic studies of TRH may help achieve better individual outcomes by optimizing drug therapy based on genetic variation.

Competing Interests

The authors declare that they have no competing interests.

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