

Original article

Parameters of vascular tone regulation and gene polymorphism associated with cardiovascular risk in young subjects

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Received 7 March 2023, Revised 18 April 2023, Accepted 4 May 2023

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Abstract: *Introduction* — The identification of preclinical stages of vascular pathology is the most promising for prevention of hypertension (HTN). It is important to investigate the polymorphism of genes which end products are involved in the regulation of blood pressure (BP) and predispose to vascular tone (VT) dysregulation.

Objective — To investigate the clinical and prognostic significance of the *AGT* and *AGTR1* polymorphic variants associated with increased cardiovascular risk in young subjects and patients with HTN.

Methods — The study involved 2 independent groups: young healthy volunteers and hypertensive patients. The VT regulation was assessed by the active standing test. The polymorphism was identified using DNA pyrosequencing.

Results — The C allele of the *AGTR1* A1666C A>C variant was associated with lower HR in supine in both groups. The risk allele C of the M268T T>C polymorphism was associated with lower systolic BP and diastolic BP during the 1st minute of upright posture. The C allele of the *AGTR1* A1666C A>C variant was associated with earlier onset of HTN.

Conclusion — The identification of the *AGTR1* A1666C A>C and *AGT* M268T T>C variants can be informative for clarifying the risk of HTN when the young subjects are examined, as well as the probability of early onset of HTN.

Keywords: genetic polymorphism, active standing test, hypertension.

Cite as Akimova NS, Elkina AYu, Dzhioeva ON, Sokolov IM, Kiselev AR, Drapkina OM, Shvarts YuG. Parameters of vascular tone regulation and gene polymorphism associated with cardiovascular risk in young subjects. *Russian Open Medical Journal* 2023; 12: e0204.

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Introduction

Dysregulation of vascular tone (VT) is one of the cardiovascular risk factors that significantly worsens the quality of life, and can be a predictor for persistent hypertension (HTN) [1]. The identification of preclinical stages of vascular pathology is the most promising for prevention of hypertension. Therefore, it is important to investigate the polymorphism of genes which end products are involved in the regulation of blood pressure (BP) and predispose to VT dysregulation. The previous studies have shown that the risk alleles of the renin-angiotensin-aldosterone system (RAAS) genes such as angiotensinogen (*AGT*) gene, angiotensin II receptor type 1 (*AGTR1*) gene are associated with more frequent development of HTN. Moreover, the risk alleles of the *AGT* and *AGTR1* genes are more common in this population compared to the healthy subjects [2-5].

The angiotensinogen (*AGT*) gene encodes the angiotensinogen protein produced by hepatocytes and involved in formation of angiotensin II which is a strong vasoconstrictor. To date, more than 15 polymorphic variants are known, most of which lead to amino acid substitutions [2]. We analyzed two *AGT* polymorphic variants: *T207M C>T (rs4762)* and *M268T T>C (rs699)*. The risk

alleles 207M and 268T are associated with increased expression of angiotensinogen and development of HTN [4].

The angiotensin II receptor type 1 (*AGTR1*) gene encodes the angiotensin II type 1 receptors locating in the vascular endothelium and mediating all major cardiovascular effects of angiotensin. Like other components of the RAAS, this gene regulates the BP [5-7]. More than 50 polymorphic variants are known. The A1666C A>C (rs5186) variant which leads to substitution of adenine for cytosine at position 1166 has the most clinical significance. The risk allele C of the A1666C A>C variant increases in sensitivity of type 1 receptors to the normal angiotensin II level, and, consequently, increases the BP. The studies have shown that the hypertensive patients significantly more often had the *AGTR1* A/C or C/C variant compared to the healthy subjects [7-10].

It should be noted that these studies mainly concerned the relationship between genetic polymorphism and BP and heart rate (HR) and the investigators usually used 24-hour Holter monitoring in these studies [11, 12]. The active standing test (AST) used in the hypertensive patients as one of the most informative and physiological tests for assessment of the VT regulation and investigation of the relationship between the BP and HR and the

AGT M268T T>C and T207M C>T, and AGTR1 A1666C A>C variants would allow to evaluate in more detail the significance of the studied polymorphic variants in VT regulation, which is of no small significance both in development and progression of cardiovascular diseases.

The investigation of the role of polymorphic variants involved in the RAAS activities will allow to understand better the structure of VT, as well as be useful in assessing the risk of HTN in relatively healthy subjects. Thus, the relevance of this study is not only practical, but also theoretical, concerning the expansion of ideas about the etiology and pathogenesis of hypertension. It should be noted that a significant part of the previous studies aimed at assessing the genetic risk of developing HTN concerns people already suffering from HTN, while information on the distribution of these polymorphic variants among people with only cardiovascular risk factors is practically absent in the literature.

Objective. To investigate the clinical and prognostic significance of polymorphic variants of the AGT and AGTR1 genes associated with increased cardiovascular risk in young and relatively healthy subjects and patients with HTN.

Material and Methods

The study involved 152 subjects, among whom there were 2 independent groups: a group of young healthy volunteers aged from 20 to 25 years (90 subjects) and a group of hypertensive patients aged from 35 to 65 years (62 subjects). The study complies with the principles of the Declaration of Helsinki. The study protocol was approved by the Ethics Committee at the Saratov State Medical University (protocol No. 3, 1 Nov 2016).

The study included only Caucasians. The exclusion criteria for young subjects were as follows: organic cardiovascular and central nervous system disorders and smoking. The exclusion criteria for patients with HTN were identical. Additionally, the patients with uncontrolled HTN were not included. To confirm the presence and controllability of HTN, we used data from ambulatory blood pressure monitoring (ABPM) performed within 2 weeks prior to enrollment in the study. The recruitment of second group was necessary to carry out a frequency analysis of the genotype, as well as to assess the "influence" of genes involved in the regulation of the renin-angiotensin-aldosterone system on BP and HR in subjects already with HTN. All patients with HTN had a normal BP range (<140 and 90 mmHg) at the time of inclusion in the study.

After signing the informed consent, the complaints and medical history were collected and physical examination was performed in all examined patients; the available medical records were reviewed. In addition to routine clinical examinations and tests, the active standing test was performed (to assess the vascular tone) and the venous blood was sampled (to identify the polymorphic variants). The choice of the active standing test for assessing the regulation of vascular tone is due to the simplicity of its implementation, availability, non-invasive technique of its implementation. The method of conducting an active standing test is based on the assessment of BP, HR, sometimes electrocardiographic data, initially in a horizontal position (the patient is lying on the couch), then in the process of being in an upright position (standing). The measurement of these parameters occurs throughout the entire period of orthostasis with an interval of 1 minute. All information was entered into a standardized

medical record. The main clinical characteristics are presented in [Table 1](#) and [Table 2](#).

It should be noted that some patients received monotherapy of HTN by beta-adrenergic blocking agents. The prescription of this class of drugs was the treatment of child-bearing potential women.

The VT regulation was assessed using the AST. The polymorphism was identified by the DNA pyrosequencing using the PyroMark Q24 Sequencer System. The characteristics of studied polymorphic variants are presented in [Table 3](#).

The statistical tests were performed using the Statistica-10 software. To process the results, non-parametric correlation analysis (Gamma factor), univariate and multivariate analyses of variance (ANOVA) were used. The multiple comparison procedure using the Kruskal-Wallis test was used for each studied polymorphism. The distribution of genotype frequencies of the studied genes was tested for the Hardy-Weinberg equilibrium using the χ^2 test.

In accordance with the objectives of the study, we purposefully recruited participants into two groups that differed significantly in age and clinical characteristics. Since one of the tasks was precisely the establishment of relationships between gene polymorphism and vascular tone, which do not depend on age. We did not compare the groups we studied in the traditional way, including no methods of comparing any quantitative characteristics. The relationships within each group were studied during the work.

Table 1. The main clinical characteristics of the young subjects

Parameter	Subjects (n=90)
Mean age, years	21.3
Male, n (%)	32 (35.6)
Female, n (%)	58 (64.4)
Obesity, n (%)	5 (5.6)
Family history*, n (%)	48 (53.3)

* – hypertension, coronary heart disease, acute cerebrovascular accident in the parents.

Table 2. The main clinical characteristics of the patients with HTN

Parameter	Subjects (n=62)
Mean age, years	51.6
Male, n (%)	24 (38.7)
Female, n (%)	38 (61.3)
Obesity, n (%)	24 (45.2)
Family history*, n (%)	58 (93.6)
HTN therapy (monotherapy or combination therapy)	
ACE inhibitors/sartans, n (%)	58 (93.6)
Selective beta-adrenergic blocking agents, n (%)	18 (29.0)
Dihydropyridine calcium antagonists, n (%)**	12 (19.4)

* – HTN, CHD, and acute cerebrovascular accident in the parents; ** – the patients received only amlodipine as a dihydropyridine calcium antagonist.

Table 3. The characteristics of the studied polymorphic variants

Gene	Protein	Polymorphism	rs	Variants
AGT	Angiotensinogen	T207M C>T	rs4762	CC; CT; TT
AGT	Angiotensinogen	M268T T>C	rs699	TT; TC; CC
AGTR1	Angiotensin II receptor type 1	A1666C A>C	rs5186	AA; AC; CC

Table 4. Frequency distribution of AGT, AGTR1 genotypes among young subjects

Gene	Polymorphism	rs	Distribution of genotypes, n (%)	
AGT	T207M C>T	rs4762	CC	59 (65.6)
			CT	30 (33.3)
			TT	1 (1.1)
			CT + TT	31 (34.4)
AGT	M268T T>C	rs699	TT	19 (21.1)
			TC	54 (60.0)
			CC	17 (18.9)
			TC + CC	71 (78.9)
AGTR1	A1666C A>C	rs5186	AA	53 (59.0)
			AC	35 (39.0)
			CC	2 (2.0)
			AC + CC	37 (41.0)

Table 5. Frequency distribution of AGT and AGTR1 genotypes among patients with HTN

Gene	Polymorphism	rs	Distribution of genotypes, n (%)	
AGT	T207M C>T	rs4762	CC	40 (64.5)
			CT	22 (35.5)
			TT	0 (0)
			CT + TT	22 (35.5)
AGT	M268T T>C	rs699	TT	8 (12.9)
			TC	42 (67.7)
			CC	12 (19.4)
			TC + CC	54 (87.1)
AGTR1	A1666C A>C	rs5186	AA	42 (67.7)
			AC	16 (25.8)
			CC	4 (6.5)
			AC + CC	20 (32.3)

Table 6. The relationship between the age of HTN onset and the AGT and AGTR1 variants.

	AGT T207M C>T	AGT M268T T>C	AGTR1 A1666C A>C
Age of HTN onset	0.124378	0.166667	0.389500*

* – statistical significance (p<0.05).

Results

Frequency distribution of AGT, AGTR1 genotypes among young subjects and patients with HTN are presented in [Tables 4](#) и [5](#). The distribution of genotype frequencies of the studied genes in young subjects and hypertensive patients was expected, given the Hardy-Weinberg equilibrium.

The BP and HR parameters measured using the AST were normal in all examined individuals. However, as expected, the patients with HTN had higher BP values. Most of the examined subjects had accelerated HR and increased diastolic blood pressure (dBP) during the 1st minute of upright posture and slightly decreased HR during the 5th minute.

The univariate and multivariate analyses revealed the relationships between the cardiac and vascular control parameters (HR, BP) and the AGTR1 A1666C A>C variant.

In particular, it was established that both in young subjects and in hypertensive patients, the 1666C variant was associated with lower HR in supine (p<0.05) ([Figure 1](#)).

In addition, the hypertensive patients with the risk allele C of this polymorphic variant had lower HR during the 1st and 3rd minutes of upright posture. Moreover, lower pulse pressure (PP) in supine and more significant decrease in dBP during the 1st, 2nd, 3rd, 4th, and 5th minutes of upright posture compared to dBP in supine

were recorded in this population (p<0,05). The change in dBP by minute is visualized in [Figure 2](#).

Relatively unexpected result was also obtained for the AGT gene. Both young subjects, and hypertensive patients who had the risk allele C of the M268T T>C variant had lower sBP and dBP during the 1st minute of upright posture (p<0,05) ([Figures 3](#) and [4](#)).

The hypertensive patients had also lower sBP during the 2nd and 3rd minutes of upright posture and lower PP during the 2nd minute of upright posture (p<0,05). Moreover, smaller increase in sBP was noted during the 4th minute of upright posture compared to sBP recorded in supine. The subjects with the risk allele also had a higher difference in PP during the 2nd minute and in supine (p<0,05).

The AGT T207M C>T variant did not have significant effect on the AST results.

It should be noted that in addition to investigation of the relationship between the hemodynamic parameters (using the active standing test) and AGT and AGTR1 genetic polymorphism, we also analyzed the association between the age of HTN onset and studied polymorphic variants. The allele C of the AGTR1 A1666C A>C variant was found to be associated with earlier onset of HTN in hypertensive patients ([Table 6](#)).

Discussion

The distribution of genotype frequencies of the studied genes is sufficiently typical according to the Hardy-Weinberg analysis. The AST parameters were also normal. This confirms that our population was representative. The genotypes in our study, for example AGT M268T T>C, have repeatedly been linked to high blood pressure. However, the effects in large GWAS studies (eg Giri et al, 2018 with 750000 individuals) show effect size of approximately 0.3 mmHg SBP for each allele. We took into account the GWAS data, and the results of our study are consistent with these global data, since the blood pressure level of the subjects at rest among individuals with the presence/absence of risk alleles of the studied genes differed slightly, which is consistent with the data of the study Giri et al. [13]. The main differences were obtained precisely during AST. It is also impossible to exclude the significance of the inclusion and exclusion criteria used in our study.

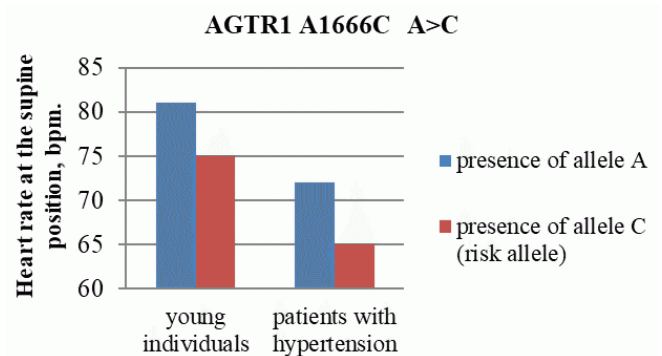


Figure 1. The heart rate in young subjects and patients with HTN in supine depending on the risk allele C of the AGTR1 A1666C A>C variant.

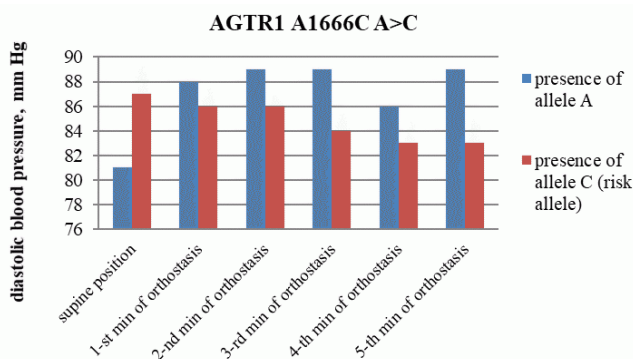


Figure 2. The diastolic BP in hypertensive patients over the time (the active standing test) depending on the risk allele of the *AGTR1* A1666C A>C variant, M±SD.

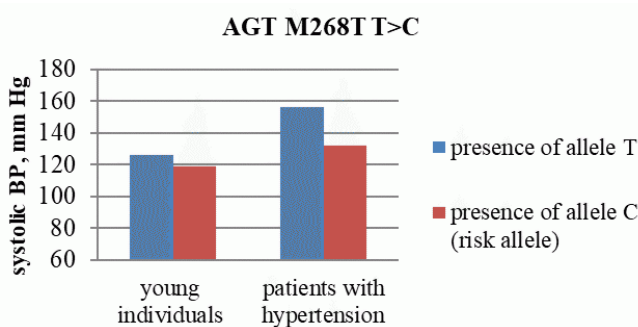


Figure 3. The systolic BP in young subjects and hypertensive patients during the 1st minute of upright posture depending on the risk allele C of the *AGT* M268T T>C variant.

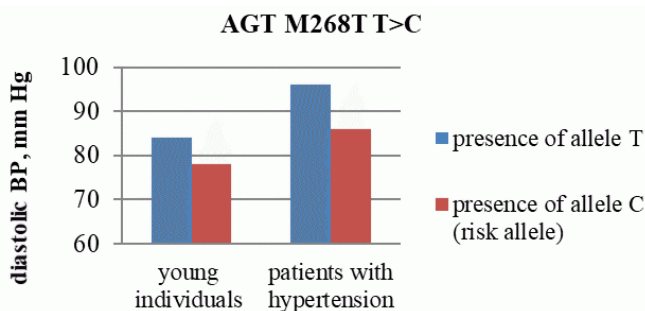


Figure 4. The diastolic BP in young subjects and hypertensive patients during the 1st minute of upright posture depending on the risk allele C of the *AGT* M268T T>C variant.

It should be noted that mean sBP and PP values in hypertensive patients were almost unchanged during the AST. It can be explained by treatment with antihypertensive drugs; moreover, the VT increased at baseline in patient with HTN can be not excluded.

The lower HR recorded by the AST in the subjects with the risk allele of the *AGTR1* A1666C A>C variant identified in the examined individuals, most likely, can be explained by decreased production of angiotensin and increased receptor sensitivity caused by this mutation, and, therefore, weaker stimulation of the sympathoadrenal system manifested by decreased HR [2]. Moreover, the obtained data can be explained both by the predominated parasympathetic regulation and by other possible

mechanisms but these hypotheses should be further investigated and confirmed.

Smaller changes in dBP in patients with the risk allele of the *AGTR1* A1666C A>C variant which contributes to greater receptor sensitivity to angiotensin, can be interpreted with decreased vascular reactivity and increased baseline vascular tone [2]. It is also possible that this result can be caused by potential compensatory decreased in the angiotensin levels and increased receptors sensitivity to angiotensin.

The lower PP values in supine in hypertensive patients with the risk allele of the *AGTR1* A1666C A>C variant are probably explained by higher systolic and diastolic BP values in supine compared to subjects without this variant [3].

The sBP and dBP features revealed during the 1st minute of upright posture in young and relatively healthy subjects and in hypertensive patients with the *AGT* M268T T>C variant may be explained both by decreased receptor sensitivity to elevated angiotensinogen levels caused by this mutation and associated decreased vascular reactivity.

The fundamental coincidence of some results of the sample of young people with the data of the examination of patients with HTN allows us to consider the identified relationships as regular, independent of age.

Therefore, the allele C of the *AGTR1* A1666C A>C variant and the allele C of the *AGT* M268T T>C variant are associated with smaller changes in BP and HR recorded during the AST in young subjects. The fundamental concordance of these results with the data obtained from the hypertensive patients allows to consider these relationships to be expected. This may suggest the direct or indirect (via the autonomic nervous system) effects of genes involved in the RAAS activities on the VT regulation, which may be manifested as increased baseline tone, and, consequently, decreased vascular reactivity. It can be assumed that identification of the studied combinations of polymorphic variants is informative as predictors for HTN and can be useful for managing the early preventive measures for HTN.

Thus, the scientific novelty of the work lies in the fact that the relationship between the polymorphic variants of the *AGT* and *AGTR1* genes and the studied hemodynamic parameters during an active orthostatic test both in young people and in patients with HTN has been established. In addition, during the study, it was found that the A1666C A>C polymorphism of the *AGTR1* gene correlates with the age of onset of HTN, on the basis of which we suggested an association of this polymorphism with an earlier development of HTN. Of course, this requires further, including prospective studies, however, this pilot work substantiates the feasibility of implementing larger projects. This pilot study justifies the feasibility of their implementation, expands the theoretical understanding of the relationship of genetic polymorphism of the studied genes with the characteristics of vascular tone.

Conclusion

Polymorphic variants of the *AGT* (rs699, rs4762), *AGTR1* (rs5186) genes are associated with the regulation of vascular tone both in young people and in patients with HTN. The presence in the genotype of patients with HTN of the risk allele C of the A1666C A>C polymorphism of the *AGTR1* gene may be associated with an earlier development of HTN.

Limitations

The main limitations of our study was the inability to conduct a classical statistical analysis of the results, as there were markedly differences between groups in many of the reported variables, mainly age. Frequency analysis of the genotype was performed in studied groups separately without comparison in the direct sense.

Informed Consent Statement

The informed consent was signed prior to the patient's enrollment.

Institutional Review Board Statement

The study complies with the principles of the Declaration of Helsinki. The study protocol was approved by the Ethics Committee at the Saratov State Medical University.

Data Availability Statement

The datasets that support the findings of this study are available from the corresponding author on reasonable request. If someone wants to request the data you should contact Anastasiya Yurievna Elkina hromyh.anastasiya@mail.ru. All data generated or analysed during this study are included in this published article.

Funding

None.

Author contributions

Conceptualization, N.S. Akimova and A.Yu. Elkina; data curation, N.S. Akimova; methodology, N.S. Akimova and Yu.G. Shvarts; validation, N.S. Akimova and I.M. Sokolov; formal analysis, A.Yu. Elkina; investigation, A.Yu. Elkina; resources, Yu.G. Shvarts; writing – original draft preparation, N.S. Akimova, A.Yu. Elkina, O.M. Drapkina, and Yu.G. Shvarts.; writing – review and editing, N.S. Akimova, A.R. Kiselev and O.M. Drapkina; visualization, A.Yu. Elkina. and O.M. Drapkina; supervision, N.S. Akimova, Yu.G. Shvarts and O.M. Drapkina; project administration, N.S. Akimova; funding acquisition, Yu.G. Shvarts. All authors have read and agreed to the published version of the manuscript.

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