Parameters of Vascular Tone Regulation and Gene Polymorphism Associated With Cardiovascular Risk in Young Subjects

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Research Article

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Abstract

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). 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.

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

Materials and methods. The study involved 90 young healthy subjects and 62 patients with hypertension. 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. The VT regulation was assessed by the active standing test in which the changes in blood pressure (BP) and heart rate (HR) were measured. The polymorphism was identified using DNA pyrosequencing.

Results. The relationship between BP and HR and the *AGTR1* A1666C A>C and *AGT* M268T T>C variants was established. Both in young subjects and hypertensive patients was found that the C allele of the *AGTR1* A1666C A>C variant was associated with lower HR in supine compared with subjects without this allele. The risk allele C of the M268T T>C polymorphism was associated with lower sBP and dBP during the 1st minute of upright posture. The identified features can probably be explained by the vascular tone increased at baseline in the presence of these polymorphic variants which was manifested by smaller changes in BP and HR during the active standing test compared to subjects without these allelic variants. 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 hypertension.

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 [2,3,4,5].

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 [5,8,9]. More than 50 polymorphic variants are known. The A1166C 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 [9,10,11,12].

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 [6,7]. 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.

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.

Materials And Methods

The study involved 90 healthy volunteers aged from 20 to 25 years and 62 hypertensive patients aged from 35 to 65 years. 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.

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. 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). All information was entered into a standardized medical record. The main clinical characteristics are presented in Table 1 and Table 2.

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 (5.56)
Family history*, n (%)	48 (53.33)

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

Table 2. The main clinical characteristics of the hypertensive patients.

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 (93.55)
HTN therapy (monotherapy or combination therapy)	ACE inhibitors/sartans, n (%)	58 (93.55)
	Selective beta-adrenergic blocking agents, n (%)	18 (29.03)
	Dihydropyridine calcium antagonists, n (%)**	12 (19.35)

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

It should be noted that some patients received beta-adrenergic blocking agents. The prescription of this class of drugs was caused by the signs of sympathicotonia or childbearing age of patients with HTN.

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.

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

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 Kruskall-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 $\chi 2$ test [13].

Results

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 (Fig. 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. 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 (Fig. 3, 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. 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.

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 4).

Table 4. 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*

*Note. * - statistical significance (p<0.05).*

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.

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. 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. 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.

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.

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.

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 hypertension.

Abbreviations

AGT – angiotensinogen gene, AGTR1 – angiotensin II receptor type 1 gene, HTN – hypertension, BP – blood pressure, dBP – diastolic blood pressure, AST – active standing test, PP – pulse pressure, sBP – systolic blood pressure, VT – vascular tone, HR – heart rate.

Declarations

Statements

Approval of ethical norms and consent to participation: 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. The informed consent was signed prior to the patient's enrollment;

Consent to publication: not applicable;

Availability of the data and materials: 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 Anastasia Yurievna Elkina hromyh.anastasiya@mail.ru. All data generated or analysed during this study are included in this published article.

Competing interests: not applicable;

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Author contribution statement

AE and NA designed the study. YuSh contributed to the statistical analysis and drafting of manuscript. AE contributed to the acquisition of data. IS critically revised the manuscript. All authors read and approved the final manuscript.

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Conflict of interest. The reported study was funded by the Saratov State Medical University according to Research Project #SSMU-2021-001.

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Figures

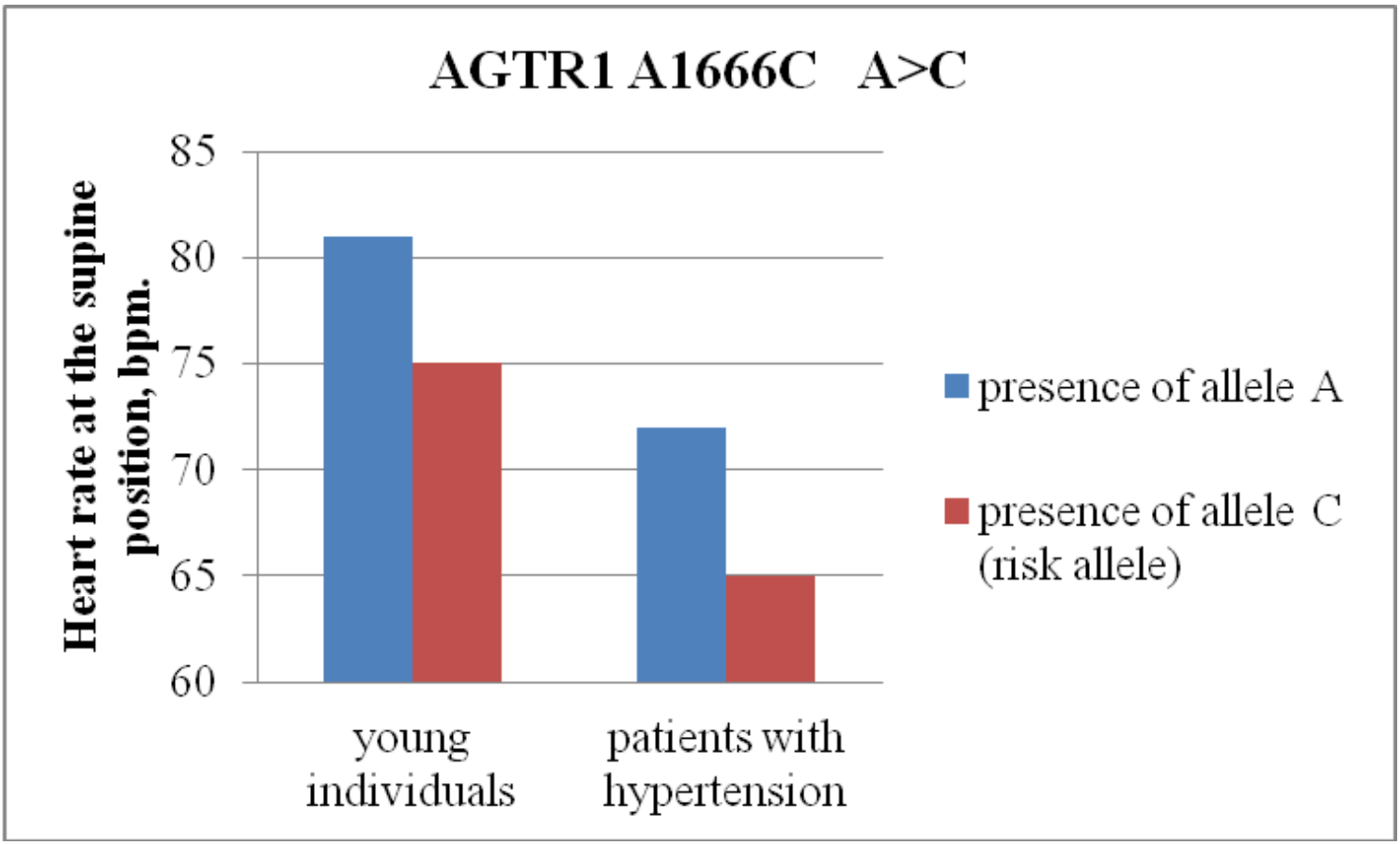

Figure 1

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

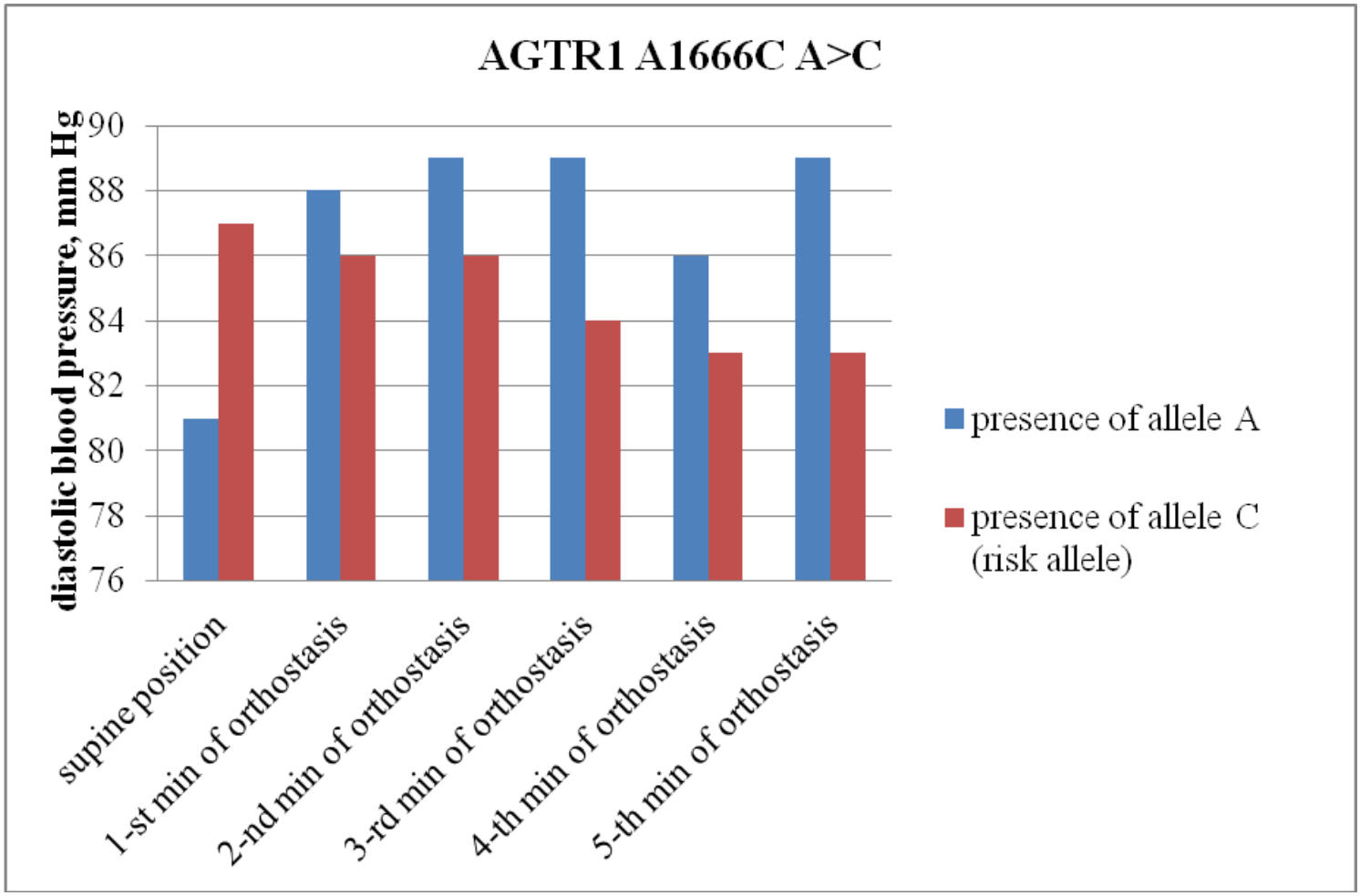

Figure 2

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.

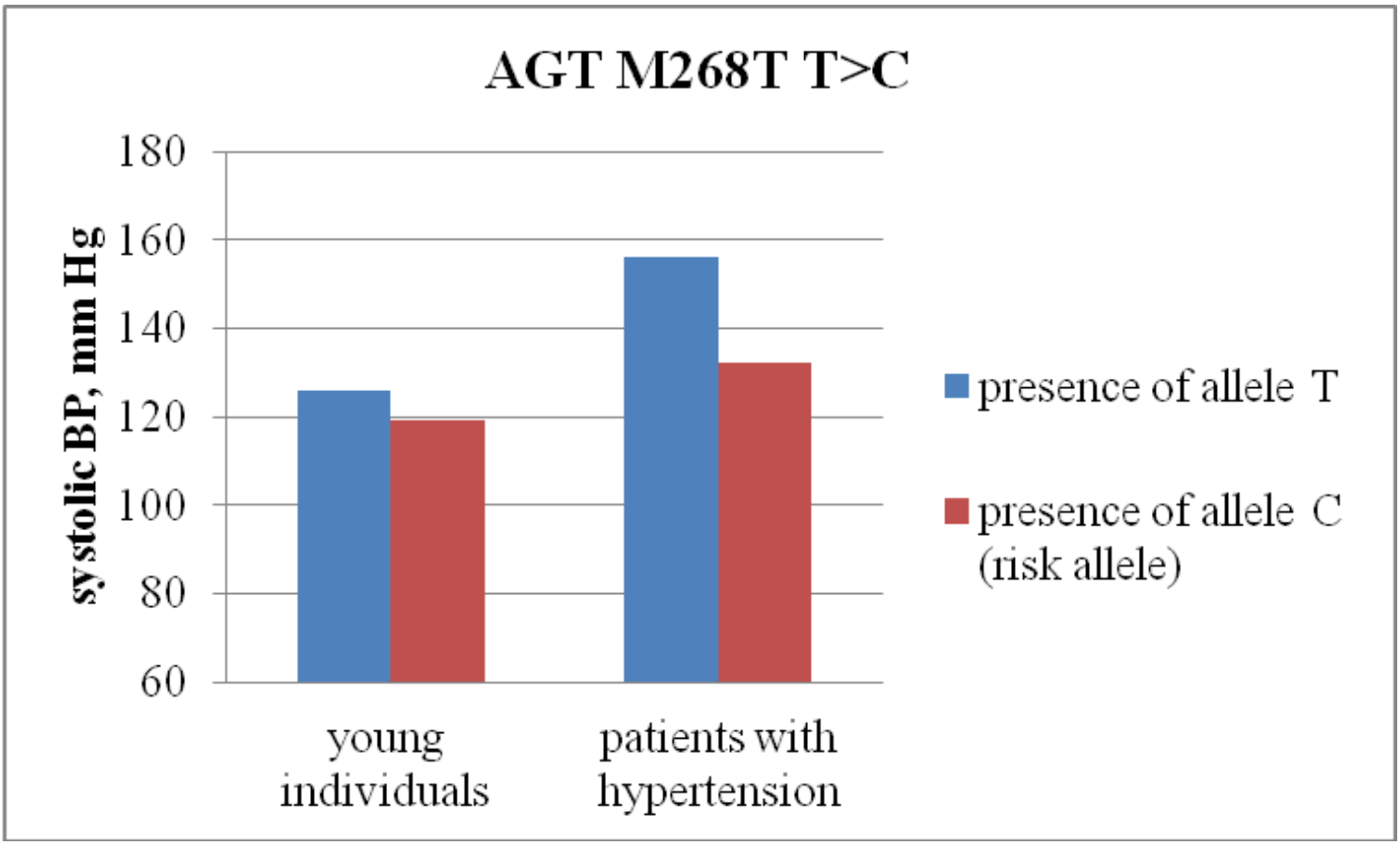

Figure 3

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.

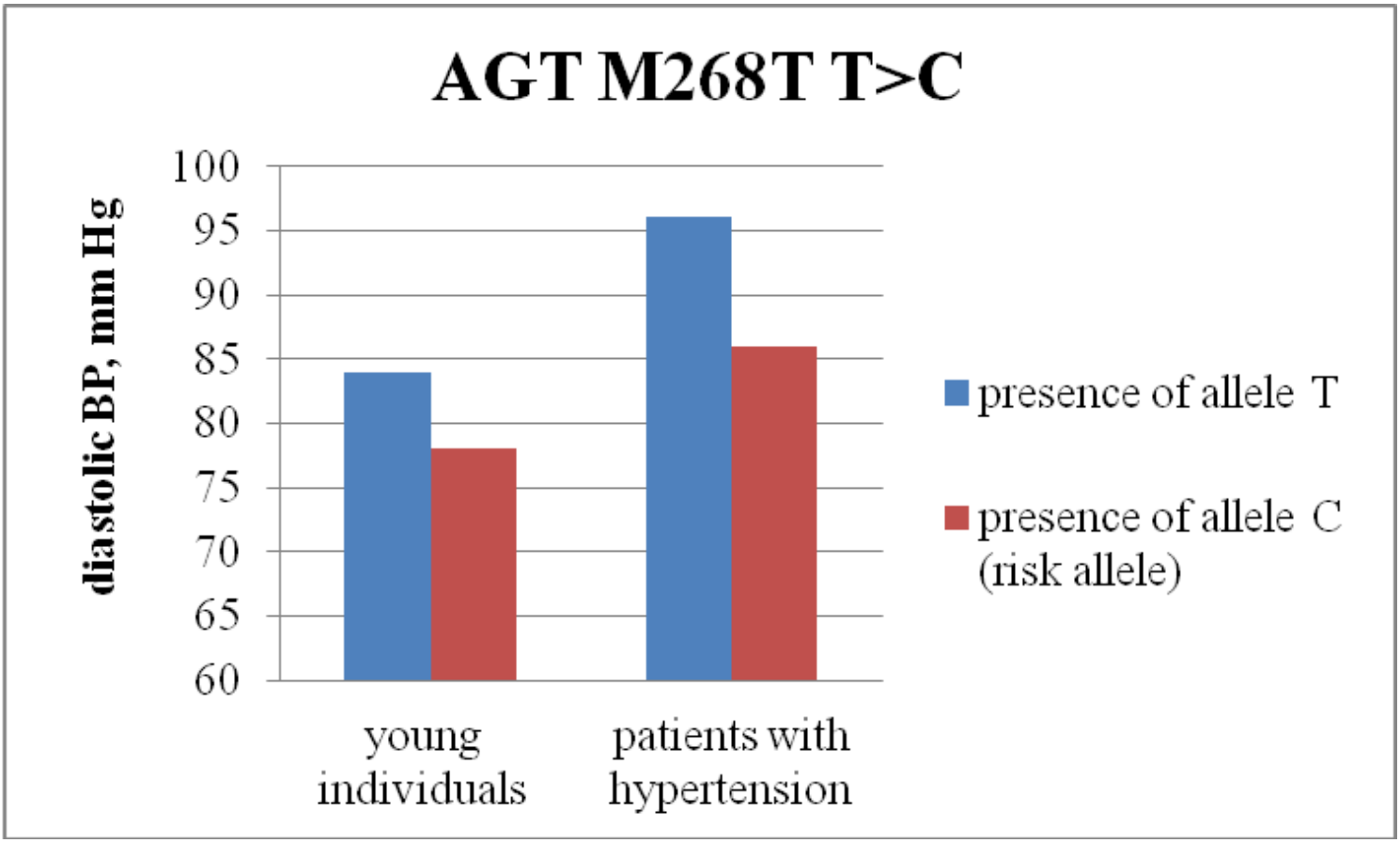

Figure 4

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.