ARCHIVES OF THE TURKISH SOCIETY OF CARDIOLOGY

Official journal of the TURKISH SOCIETY OF CARDIOLOGY

Association of the Single Nucleotide Polymorphisms in the Renin-Angiotensin-Aldosterone System with Hypertension in the Uzbek Population

Özbek Popülasyonunda Renin-Anjiyotensin-Aldosteron Sistemindeki Tek Nükleotid Polimorfizmlerinin Hipertansiyon ile İlişkisi

ABSTRACT

Objective: This research aims to identify the association between the nine polymorphic variants (rs4961, rs699, rs4762, rs5186, rs1403543, rs1799998, rs5443, rs2070744, rs1799983) and the occurrence of hypertension and its clinical manifestations in the Uzbek population.

Methods: The study included 227 individuals, comprising 179 patients with hypertension and 48 controls. Clinical parameters such as age, weight, blood glucose, triglycerides, total cholesterol, low-density lipoprotein and high-density lipoprotein, blood urea nitrogen, creatinine, pulse wave velocity, left ventricular mass, and microalbuminuria levels were identified. We assessed the distribution of allele frequencies of these polymorphic variants in the Uzbek population to establish their association with cardiovascular diseases and their clinical manifestations.

Results: Genetic analysis of the polymorphic variants demonstrated a significant association of the *AGT* 521 C>T variant with arterial hypertension [$P \leq 0.01$; Odds Ratio (OR) = 2.91]. The NOS3 -786 T>C variant correlated with left ventricular hypertrophy ($P \leq 0.05$; OR = 0.35) and increased pulse wave velocity ($P \leq 0.01$; OR = 0.21). The correlations of the *AGTR2* 1675 G>A variant with left ventricular hypertrophy ($P \leq 0.01$; OR = 1.59) and increased pulse wave velocity ($P \leq 0.01$; OR = 0.33) were identified. The *AGT* 704 T>C variant showed a significant association with increased pulse wave velocity ($P \leq 0.05$; OR = 2.73).

Conclusion: Four of the nine studied polymorphic variants were associated with clinical manifestations of hypertension in the Uzbek population. These variants can be used as genetic biomarkers to identify the risks of developing cardiovascular diseases and hypertension in the Uzbek population.

Keywords: Hypertension, single nucleotide polymorphism, pulse wave velocity

ÖZET

Amaç: Bu araştırma, Özbek popülasyonunda dokuz polimorfik varyant (rs4961, rs699, rs4762, rs5186, rs1403543, rs1799998, rs5443, rs2070744, rs1799983) ile hipertansiyon oluşumu ve klinik belirtileri arasındaki ilişkiyi belirlemeyi amaçlamaktadır.

Yöntem: Çalışmaya 179 hipertansiyon hastası ve 48 kontrol olmak üzere 227 kişi dahil edildi. Yaş, kilo, kan şekeri, trigliserit, total kolesterol, düşük yoğunluklu lipoprotein ve yüksek yoğunluklu lipoprotein, kan üre azotu, kreatinin, nabız dalga hızı, sol ventrikül kütlesi ve mikroalbüminüri düzeyleri gibi klinik parametreler belirlendi. Bu polimorfik varyantların alel frekanslarının Özbek popülasyonundaki dağılımını değerlendirerek kardiyovasküler hastalıklar ve bunların klinik belirtileri ile ilişkileri belirlendi.

Bulgular: Polimorfik varyantların genetik analizi *AGT* 521 C>T varyantının arteriyel hipertansiyon ile anlamlı bir ilişkisi olduğunu gösterdi [$P \leq 0,01$; Odds Oranı (OR) = 2,91]. NOS3 -786 T>C varyantının sol ventrikül hipertrofisi ($P \leq 0,05$; OR = 0,35) ve artmış nabız dalga hızı ($P \leq 0,01$; OR = 0,21) ile ilişkili olduğu bulundu. *AGTR2* 1675 G>A varyantının sol ventrikül hipertrofisi ($P \leq 0,01$; OR = 1,59) ve artmış nabız dalga hızı ($P \leq 0,01$; OR = 0,33) ile korelasyonu tespit edildi. *AGT* 704 T>C varyantı artmış nabız dalga hızı ile anlamlı bir ilişki göstermekteydi ($P \leq 0,05$; OR = 2,73).

Sonuç: Çalışılan dokuz polimorfik varyanttan dördü Özbek popülasyonunda hipertansiyonun klinik belirtileriyle ilişkilendirilmiştir. Bu varyantlar, Özbek popülasyonunda kardiyovasküler hastalıklar ve hipertansiyon gelişme risklerini belirlemek için genetik biyobelirteçler olarak kullanılabilir.

Anahtar Kelimeler: Hipertansiyon, tek nükleotid polimorfizmi, nabız dalga hızı

ORIGINAL ARTICLE
KLİNİK ÇALIŞMA

Darya Vladimirovna Zakirova[1]
Alisher Abdullaev[1]
Guzal Abdullaeva[2]
Zaringiz Mashkurova[2]
Elina Aguryanova[1]
Fozilakhon Omonova[1]
Sevara Bekmetova[2]

[1]*Center for Advanced Technologies, Tashkent, Uzbekistan*
[2]*Republican Specialized Research and Practical Medical Center for Cardiology, Tashkent, Uzbekistan*

Corresponding author:
Darya Vladimirovna Zakirova
✉ catdasha@mail.ru

Received: June 09, 2023
Accepted: December 26, 2023

Cite this article as: Zakirova DV, Abdullaev A, Abdullaeva G, et al. Association of the single nucleotide polymorphisms in the renin-angiotensin-aldosterone system with hypertension in the Uzbek population. *Turk Kardiyol Dern Ars.* 2024;52(3):182-188.

DOI:10.5543/tkda.2023.67866

Cardiovascular diseases (CVD) are among the main causes of morbidity and mortality worldwide,[1] primarily due to the high prevalence of hypertension (HTN) and coronary heart disease (CHD).

The renin-angiotensin-aldosterone system (RAAS) is a system of enzymes and hormones that regulate blood pressure, electrolyte balance, and water balance in the body.[2] The angiotensinogen gene (*AGT*), which encodes angiotensinogen, has two known polymorphic variants: *AGT* 704 T>C (Met235Thr) rs699 and *AGT* 521 C>T (Thr174Met) rs4762. They are located in the promoter region in non-equilibrium close linkage, affecting the operation of the entire gene. Many studies have reported that these markers are associated with HTN.[3,4]

Other genes in the RAAS are the *AGTR1* and *AGTR2* genes, which encode angiotensin II type 1 and angiotensin II type 2 receptors, respectively. Several studies have demonstrated a significant association of the *AGTR1* gene 1166 A>C (rs5186) and AGTR2 1675 G>A rs1403543 polymorphisms with HTN.[5,6]

Aldosterone synthase *CYP11B2*, a rate-limiting enzyme involved in the biosynthesis of aldosterone has a notable polymorphism, *CYP11B2* -344 C>T rs1799998. This polymorphism was found to be associated with susceptibility to HTN in Chinese populations.[7]

In addition to the RAAS system, cellular signal transduction plays an important role in regulating blood pressure as it affects cellular proliferation. The *GNB3* gene encodes heterotrimeric G-proteins, which regulate various cellular functions. The *GNB3* 825 C>T rs5443 polymorphism is associated with the loss of one domain in the protein, leading to its increased activation and resulting in vasoconstriction.[8,9]

With an insufficient amount of nitric oxide (NO) in the blood, encoded by the *NOS3* gene, the arterial lumen narrows, increasing blood pressure.[10] An association of the *NOS3* -786 T>C (rs2070744) and *NOS3 G894T* (rs1799983) polymorphisms with HTN has been found in many studies.[11,12]

Since the distribution of allele and mutation frequencies in genes varies based on geographical and ethnic characteristics,[13] this study aims to establish the association of these polymorphisms with HTN and its clinical presentations in the Uzbek population.

ABBREVIATIONS

AA	Aortic Augmentation
AH	Arterial Hypertension
AIx	Central Augmentation Index
BMI	Body Mass Index
BP	Blood Pressure
BUN	Blood Urea Nitrogen
CAD	Coronary Artery Disease
cDBP	Central Diastolic Blood Pressure
CHD	Coronary Heart Disease
CI	Confidence Interval
cPP	Central Pulse Pressure
cSBP	Central Systolic Blood Pressure
CVD	Cardiovascular Disease
EHTN	Essential Hypertension
HDL	High-Density Lipoprotein
HTN	Hypertension
IVSd	Interventricular Septal Thickness at End-Diastole
LDL	Low-Density Lipoprotein
LVEDD	Left Ventricular End-Diastolic Dimension
LVESD	Left Ventricular End-Systolic Dimension
LVH	Left Ventricular Hypertrophy
LVM	Left Ventricular Mass
LVMI	Left Ventricular Mass Index
PW	Pulse Wave
PWd	Posterior Wall Thickness at End-Diastole
PWV	Pulse Wave Velocity
RAAS	Renin-Angiotensin-Aldosterone System
RSSPMCC	The Republican Specialized Scientific and Practical Medical Center of Cardiology
SD	Standard Deviation
TC	Total Cholesterol
TG	Triglycerides

Materials and Methods

This study included 227 individuals, of which 179 were patients with Stage 1 to Stage 3 HTN according to the classification by the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC), 2018, and 48 were controls. Patients were receiving outpatient care at the Republican Specialized Scientific and Practical Medical Center of Cardiology (RSSPMCC) under the Ministry of Healthcare of the Republic of Uzbekistan.

This research was conducted within the framework of the project FZ-201811215, supported by the Ministry of Innovative Development of Uzbekistan. It was approved by the Center For Advanced Technologies Ethics Committee at the Republican Specialized Scientific and Practical Medical Center of Cardiology (Approval Number: 201811215, Date: 07.04.2021), and all study participants provided informed consent. The study was conducted in accordance with the World Medical Association Declaration of Helsinki (WMA, 2013).

Patient Selection

The exclusion criteria were as follows: symptomatic arterial hypertension, Class III and IV angina pectoris, Functional Class III-IV (FC III-IV) of chronic heart failure, cardiac arrhythmias, classes I, II, III unstable angina according to the Braunwald clinical classification, history of ischemic stroke or myocardial infarction, complications induced by diabetes mellitus, inherited metabolic disorders, renal and hepatic insufficiency, and oncologic diseases. Venous blood sampling and clinical studies of patients and the control group were conducted at the RSSPMCC. Main clinical parameters such as age, weight, blood glucose, triglycerides (TG), total cholesterol (TC), low-density lipoproteins (LDL) and high-density lipoproteins (HDL), blood urea nitrogen (BUN), creatinine, pulse wave velocity (PWV), left ventricular mass, and microalbuminuria levels were identified. HTN was diagnosed in accordance with the recommendations (ESH/ESC, 2018).

Applanation Tonometry

Applanation tonometry was performed on patients using the SphygmoCor apparatus (AtCor Medical, Sydney, Australia). Using the software, the pulse contour method was applied to analyze peripheral and central pulse wave (PW) with the calculation of peripheral and central blood pressure (BP), taking into account the brachial BP (systolic BP [SBP], diastolic BP [DBP], pulse pressure [PP]), and other parameters characterizing vascular elasticity and vascular tone. The following indicators of augmentation were determined using the SphygmoCor apparatus: aortic augmentation (AA), central systolic BP (cSBP), central diastolic

BP (cDBP), central PP (cPP), and central augmentation index (AIx). Moreover, an additional augmentation index is calculated based on the heart rate (HR) at 75 beats per minute - AIx@HR75. To assess the propagation PW velocity (PWV), the distance (in mm) is measured between the site of pulsation of the femoral artery under the inguinal ligament and the clavicle (distal distance), and the distance (in mm) between the site of pulsation of the common carotid artery in the carotid triangle and the clavicle (proximal distance). Next, three electrodes are placed on the upper limbs and the left leg, and the PW is sequentially recorded on the common carotid and femoral arteries with simultaneous electrocardiogram (ECG) recording. Then automatic calculations of PWV are performed.[14]

Echocardiographic Assessment

The echocardiographic assessment was performed using the ultrasound machine EnVisor C (PHILIPS, Amsterdam, Netherlands) in accordance with the recommendations of the American Society of Echocardiography using M- and B-modes.[15] In M-mode, measurements were obtained through the parasternal axis view in accordance with the recommendations of the Penn Convention method.[16] The following parameters of intracardiac hemodynamics were studied: left ventricular end-diastolic dimensions (LVEDD) and left ventricular end-systolic dimensions (LVESD); left ventricular wall thickness: interventricular septal thickness at end-diastole (IVSd) and posterior wall thickness at end-diastole (PWd). Left ventricular mass was calculated using the formula by Devereux B.R. and co-author.[16] Left Ventricular Mass (LVM)=1.04×[(LVEDD+IVSd+PWd)^3-LVEDD^3]-13.6(g). Left Ventricular Mass Index (LVMI) was calculated using this formula: LVMI=LVM/body surface area. Left Ventricular Hypertrophy (LVH) was diagnosed in cases with LVMI>115 g/m for males and >95 g/m for females.[17]

Genotyping

Polymorphic variants of the genes were identified via real-time polymerase chain reaction using allele-specific primers, gene-specific primers, and Deoxyribonucleic Acid (DNA) probes. Variants studied included: *ADD1 G1378T* (rs4961), *AGT T704C* (rs699), *AGT C521T* (rs4762), *AGTR1 A1166C* (rs5186), *AGTR2 G1675A* (rs1403543), *CYP11B2 C*(-344)T (rs1799998), *GNB3 C825T* (rs5443), *NOS3 T* (-786)*C* (rs2070744), *NOS3 G894T* (rs1799983).

Statistical Analysis

Clinical parameters and genotyping data were entered into Microsoft Excel 2019 (Microsoft, Redmond, Washington, USA) for initial processing. The interquartile range (lower quartile – 25%; upper quartile – 75%) and the median of the sample were calculated. Logistic regression analysis was conducted using the R programming language (R Core Team, Indianapolis, Indiana, USA) and the SNPassoc software package (The R Foundation for Statistical Computing, Vienna, Austria) to analyze statistically significant correlations between the predicted genotype and the development of the disease via common genetic models.

Results

The ages of hypertensive patients participating in the research ranged from 26 to 71, consisting of 107 females (59.8%) and 72 males (40.2%). The ages in the control group ranged from 44 to 71, with 32 females (66.7%) and 16 males (33.3%). According to the Mann-Whitney U tests, the age difference between the hypertensive patients and the control group was not statistically significant (P = 0.051, U = 1.95). The difference in body mass index (BMI) was statistically significant between the group of HTN patients and the control group (Table 1). As shown in Table 1, blood glucose levels also showed a statistically significant difference between the two groups (Figure 1). TG levels in the cases were also significantly different from those in the control group (Table 1). In the Uzbek population, the difference in serum TC between the group of HTN patients and the control group was not statistically significant, although the median serum TC level was slightly higher among the cases (Table 1). HDL levels

Table 1. Investigation of the Clinical Data and Medical History According to the Mann-Whitney U Tests

Clinical Data	Cases (HTN Patients) Median (QL, QU)	Controls (Healthy) Median (QL, QU)	Mann-Whitney U	
			P	Value *U*
BMI (kg/m^2)	31 (28, 34.75)	27.6 (25, 29)	0.00001	494.595
Blood Glucose (mmol/L)	5 (5, 6)	5.2 (4.975, 5.45)	0.00001	414.599
TG (mg/dL)	147 (110, 211)	100 (72.25, 144.25)	0.0001	386.887
TC (mg/dL)	195 (164.25, 224.5)	184 (170, 219.5)	0.76418	0.2975
HDL-C (mg/dL)	43 (37, 51)	50.5 (41, 65)	0.00194	309.981
LDL-C (mg/dL)	109 (70.5, 135.25)	103 (92.5, 140.5)	0.63836	0.47291
BUN (mmol/L)	6 (5, 7.25)	5.2 (4, 6)	0.01208	250.865
Creatinine (mmol/L)	87 (71, 100.75)	73.5 (64.5, 86.75)	0.00466	282.504
PWV (m/s)	10 (8.9, 12.8)	7.9 (6.75, 8.9)	0.00128	321.999
LVMI (g/m^2)	125 (105, 161.25)	70.5 (63.5, 78)	0.00001	866.249
Microalbuminuria (mg/mL)	16 (6.75, 33)	21 (11, 42.5)	0.22628	121.031

BMI, Body Mass Index; BUN, Blood Urea Nitrogen; HDL-C, High-Density Lipoprotein-Cholesterol; HTN, Hypertension; LDL-C, Low-Density Lipoprotein-Cholesterol; LVMI, Left Ventricular Mass Index; PWV, Pulse Wave Velocity; QL, Lower Quartile (25%); QU, Upper Quartile (75%); TC, Total Cholesterol; TG, Triglyceride.

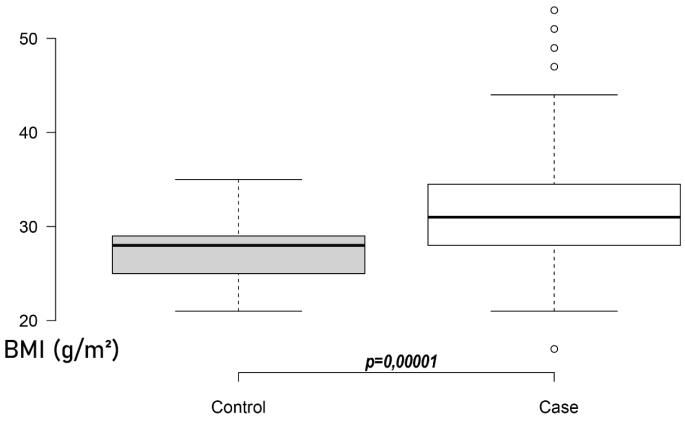

Figure 1. Distribution of Body Mass Index (BMI) in controls and cases (hypertensive patients).

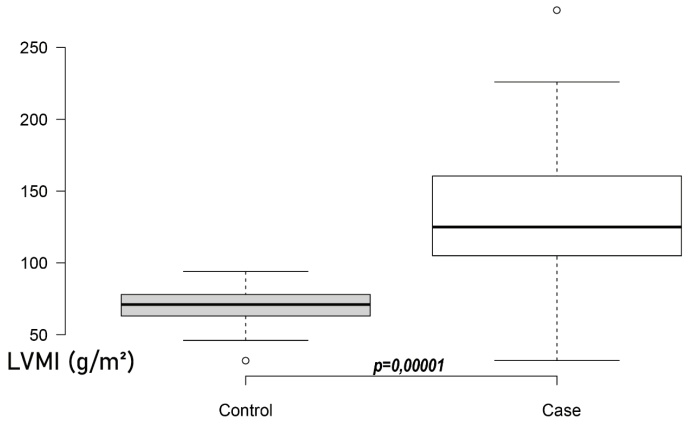

Figure 2. Distribution of Left Ventricular Mass Index (LVMI) in controls and hypertensive patients.

Table 2. Genotype and Allele Frequencies in Two Groups

Gene	Polymorphic Variant	n		Genotype Frequency						Allele Frequency			
				Common Homozygotes		Heterozygotes		Rare Homozygotes		Dominant Alleles		Recessive Alleles	
		Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
ADD1	rs4961	179	48	0.60	0.52	0.33	0.46	0.08	0.02	0.76	0.75	0.24	0.25
AGT	rs4762	178	45	0.69	0.87	0.27	0.13	0.04	0.00	0.82	0.93	0.18	0.07
AGT	rs699	177	36	0.19	0.19	0.46	0.42	0.35	0.38	0.42	0.40	0.58	0.60
AGTR1	rs5186	178	48	0.75	0.75	0.23	0.23	0.02	0.02	0.87	0.83	0.13	0.17
AGTR2	rs1403543	179	48	0.38	0.23	0.2	0.42	0.35	0.35	0.52	0.44	0.48	0.56
CYP11B2	rs1799998	176	48	0.27	0.29	0.46	0.37	0.27	0.33	0.50	0.48	0.50	0.52
GNB	rs5443	176	47	0.43	0.40	0.44	0.51	0.13	0.08	0.65	0.66	0.35	0.34
NOS3	rs2070744	179	36	0.56	0.56	0.35	0.36	0.09	0.08	0.74	0.74	0.26	0.26
NOS3	rs1799983	175	48	0.60	0.60	0.33	0.32	0.07	0.07	0.76	0.78	0.24	0.22

were significantly higher in the control group compared to the cases, while LDL levels did not show significant differences (Table 1). BUN levels and creatinine were considerably increased among the HTN patients (Table 1). Parameters such as PWV and LVMI were greater (statistically significant) in the group of hypertensive patients (Figure 2).

Results of the genetic analysis of polymorphic gene variants in the groups of cases and controls in the Uzbek population demonstrated a correlation between the *AGT* 521 C>T variant and HTN according to codominant (P = 0.047), additive (P = 0.047), and dominant genetic models (P = 0.01) (Table 3). Genotype frequency distribution in both groups corresponded to the Hardy-Weinberg equilibrium (P > 0.05). While studying the association with LVH, the cases group consisted of HTN patients with diagnosed LVH, whereas the control group consisted of HTN patients without LVH and a healthy group. Based on a significant correlation in the recessive genetic model, LVH was associated with the *NOS3 -786* T>C polymorphic variant (P = 0.04) (Table 3). It is also worth noting that the *NOS3 -786* T>C polymorphic variant correlates with increased PWV in terms of codominant (P = 0.03), additive (P = 0.02), and recessive (P = 0.01) genetic models, as shown in Table 3. Genotype frequency distribution in both groups corresponded to the Hardy-Weinberg equilibrium (P > 0.05). Our study revealed a statistically significant association of the *AGTR2* 1675 G>A polymorphic variant with LVH in terms of codominant (P = 0.004) and recessive (P = 0.010) genetic models (Table 3). The distribution of genotype frequencies corresponded to the Hardy-Weinberg equilibrium only in the control group (P > 0.05). Furthermore, an association was found between *AGTR2* 1675 G>A and increased PWV (>10 m/s) in the codominant (P = 0.004), additive (P = 0.01), and dominant (P = 0.002) genetic models (Table 3). The distribution of genotype frequencies did not correspond to the Hardy-Weinberg equilibrium in either of the groups (P < 0.05). Moreover, the presence of the A allele has a protective effect in cases of both LVH and increased PWV.

An association was found between *AGT* 704 T>C and increased PWV (>10 m/s) in the recessive genetic model (P = 0.03) (Table 3). The genotype frequency distribution corresponded to the Hardy-Weinberg equilibrium in both groups (P > 0.05) (Table 3).

Table 3. Inheritance of Arterial Hypertension, Left Ventricular Hypertrophy, and Pulse Wave Velocity Using Different Models

Polymorphic Variant		*AGT (C521T)* rs 4762		*AGT (T704C)* rs rs699		*AGTR2 (G1675A)* rs1403543				*NOS3 (T-786C)* rs2070744			
Clinical Parameter		**HTN**		**PWV**		**PWV**		**LVH**		**LVH**		**PWV**	
		OR	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
Codominant Model	Common Homozygotes	1.00	-	NS	NS	1.00	-	1.00	-	NS	NS	1.00	-
	Heterozygotes	2.48	0.99-6.25*	NS	NS	0.25	0.10-0.63	0.50	0.25-0.97	NS	NS	0.76	0.38-1.52*
	Rare Homozygotes	0.00	-	NS	NS	0.39	0.18-0.85**	1.59	0.79-3.19**	NS	NS	0.19	0.05-0.73
Additive Model		2.83	1.20-6.70*	NS	NS	0.62	0.42-0.91*	NS	NS	NS	NS	0.56	0.33-0.93*
Dominant Model		2.91	1.16-7.27*	NS	NS	0.33	0.16-0.66**	0.40	0.22-1.73	NS	NS	NS	NS
Recessive Model		NS	NS	2.73	1.09-6.83*	NS	NS	2.20	1.19-4.08**	0.35	0.12-0.97*	0.21	0.05-0.79*

*value $P \leq 0.05$; **value $P \leq 0.01$.
CI, Confidence Interval; HTN, Hypertension; LVH, Left Ventricular Hypertrophy; NS, Not Statistically Significant; OR, Odds Ratio; PWV, Pulse Wave Velocity.

The study of the association of BMI and LVMI with the polymorphic variants that we investigated did not show statistically significant values with any of the examined markers ($P > 0.05$).

Discussion

Our results showed that metabolic syndrome, diabetes mellitus, and hypertension are closely associated and often found together, as confirmed by other studies.[18] Additionally, it is known that hypertension and the progression of atherosclerosis are two pathological processes that complement each other. This was demonstrated in our study, where the levels of triglycerides differed significantly between the cases and the control group ($P < 0.001$).[19] According to the literature, the level of serum oxidative stress is a predictor of mortality in the context of cardiovascular disease.[20] However, in the Uzbek population, differences between the cases and control groups were not statistically significant. This may be because oxidative stress depends not only on the total amount of cholesterol in the blood but also on its ratio with different forms of lipoproteins. It is widely known that elevated levels of LDL are associated with the early development of CVDs, while a decrease in LDL is linked to an increase in anti-atherogenic HDL levels.[21,22] However, our study showed statistically significant differences only in terms of HDL levels, which were higher in the control group than in the cases. BUN levels and creatinine were significantly increased among HTN patients, which aligns with other experimental and clinical studies. These studies demonstrate that HTN and proteinuria are not only complementary markers but also key pathophysiological mechanisms underlying chronic kidney disease progression.[23-25] Parameters such as PWV and LVMI were greater (statistically significant) in the group of hypertensive patients. This can be explained by the long-term effect of common cardiovascular risk factors. Changes in microcirculation caused by the long-term effects of HTN lead to persistent spasms of arterioles, thickening of arterial walls, and reduction of microvasculature[26] (Figure 2).

According to the results of our genetic analysis, the *AGT* 521 C>T variant correlates with hypertension under several genetic models ($P < 0.05$). The *AGT* 521 C>T variant is associated with angiotensinogen levels in the blood. When cytosine is replaced by thymine, the amino acid threonine is replaced by methionine in the 207th position. The presence of the T allele in a genotype increases the expression of angiotensinogen and the risk of developing hypertension.[27] The frequency of the T allele in different populations ranges from 29% in the Gujarati Indian population in Houston, Texas, USA, to 5.5% in the population of African descent in the American Southwest.[28]

A statistically significant correlation was found between LVMI and the NOS3 -786 T>C polymorphic variant ($P < 0.05$). We hypothesize that this correlation is due to the fact that the production of nitric oxide synthases is regulated by eNOS (endothelial NOS NO synthase), which is encoded by the NOS3 gene located on chromosome 7q35-36 in vascular endothelium and cardiomyocytes.[29] In addition to its vasodilating effect, which reduces cardiac workload, NO can also stimulate angiogenesis, reduce the hypertrophy of cardiomyocytes, limit the production of extracellular matrix proteins by cardiac fibroblasts, and is associated with the pathophysiology of left ventricular remodeling.[30,31] Our results, which found a correlation of the polymorphic variant NOS3 -786 T>C with LVH and elevated PWV simultaneously, are consistent with studies by Romanian scientists. They stated that PWV can be a marker of vascular remodeling. Moreover, increased PWV significantly correlates ($P < 0.002$) with the presence of cardiac disorders, defined as the presence of concentric remodeling or LVH.[32]

We identified a statistically significant correlation ($P < 0.01$) while studying the relationship between the *AGTR2* 1675 G>A polymorphic variant and LVH. Furthermore, we found an association between this polymorphism and increased PWV (>10 m/s) ($P < 0.01$). Notably, the presence of the A allele has a protective effect in both the case of LVH and increased PWV. Our results turned out to be similar to those of German scientists, who showed that the thickness of the interventricular septum is significantly lower in carriers of the A allele of the *AGTR2* 1675 G>A rs1403543 variant ($P = 0.002$),[33] and to findings from South African scientists, who also found a significant association between this polymorphism and LVH, with each A allele reducing the mean wall thickness by approximately 0.5 mm ($P = 0.02$). The *AGT* 704 T>C polymorphic variant also showed a significant association with increased PWV ($P < 0.05$). Previously, various researchers have shown that the cytosine-cytosine (CC)

homozygous variant of the allele is associated with increased levels of AGT in plasma and high blood pressure.[34,35]

This study has potential limitations, such as a small sample size. Therefore, for our findings to be generalizable to the broader population, further research with larger sample sizes should be conducted. Another limitation is that the sample in our study consisted not only of representatives of the ethnic Uzbek population but also several other ethnicities who are citizens of Uzbekistan. This means that our study does not completely reflect the distribution and associations of the studied Single Nucleotide Polymorphisms (SNPs) in the ethnic Uzbek population.

Conclusion

We confirmed that four out of the nine studied polymorphic variants demonstrated a significant correlation with HTN and its clinical manifestations in the Uzbek population. These variants are *AGT 704* T>C, *AGT 521* C>T, *AGTR2 1675* G>A, and *NOS3 -786* T>C. These polymorphic variants can potentially be used as informative genetic biomarkers to identify the risks of developing cardiovascular disease and hypertension, thereby improving the diagnosis and quality of treatment for patients with HTN in the Uzbek population.

Ethics Committee Approval: It was approved by the Center For Advanced Technologies Ethics Committee at the Republican Specialized Scientific and Practical Medical Center of Cardiology (Approval Number: 201811215, Date: 07.04.2021).

Informed Consent: All study participants provided informed consent.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - A.A.; Design - D.Z., A.A., G.A.; Supervision - D.A., A.A.; Resource - A.A.; Materials - Z.M., S.B.; Data Collection and/or Processing - Z.M., S.B., E.A., F.O.; Analysis and/or Interpretation - D.Z., A.A.; Literature Review - D.Z., A.A.; Writing - D.Z.; Critical Review - A.A., G.A.

Use of AI for Writing Assistance: No AI-assisted technology was used in this study.

Conflict of Interest: Authors declare that they have no conflict of interest.

Funding: This research was founded by Innovative Development Agency under the Ministry of Higher Education, Science and Innovation.

References

1. Skaggs BJ, Hahn BH, McMahon M. Accelerated atherosclerosis in patients with SLE--mechanisms and management. *Nat Rev Rheumatol*. 2012;8(4):214-223. [CrossRef]
2. Lu H, Cassis LA, Kooi CW, Daugherty A. Structure and functions of angiotensinogen. *Hypertens Res.* 2016;39(7):492-500. Erratum in: *Hypertens Res*. 2016;39(11):827. [CrossRef]
3. Pan WH, Chen JW, Fann C, Jou YS, Wu SY. Linkage analysis with candidate genes: the Taiwan young-onset hypertension genetic study. *Hum Genet.* 2000;107(3):210-215. [CrossRef]
4. Rankinen T, Gagnon J, Pérusse L, et al. AGT M235T and ACE ID polymorphisms and exercise blood pressure in the HERITAGE Family Study. *Am J Physiol Heart Circ Physiol.* 2000;279(1):H368-H374. [CrossRef]
5. Chandra S, Narang R, Sreenivas V, Bhatia J, Saluja D, Srivastava K. Association of angiotensin II type 1 receptor (A1166C) gene polymorphism and its increased expression in essential hypertension: a case-control study. *PLoS One*. 2014;9(7):e101502. [CrossRef]
6. Zivkovi´c M, Djuri´c T, Stanci´c O. et al. X-linked angiotensin II type 2 receptor gene polymorphisms-1332A/G in male patients with essential hypertension. *Clin Chim Acta.* 2007;386(1-2):110–113. [CrossRef]
7. Cheng X, Xu G. Association between aldosterone synthase CYP11B2 polymorphism and essential hypertension in Chinese: a meta-analysis. *Kidney Blood Press Res.* 2009;32(2):128-140. [CrossRef]
8. Siffert W, Rosskopf D, Siffert G, et al. Association of a human G-protein beta3 subunit variant with hypertension. *Nat Genet*. 1998;18(1):45-48. [CrossRef]
9. Dusse F, Frey UH, Bilalic A, et al. The GNB3 C825T polymorphism influences platelet aggregation in human whole blood. *Pharmacogenet Genomics.* 2012;22(1):43-49. [CrossRef]
10. Forte P, Copland M, Smith LM, Milne E, Sutherland J, Benjamin N. Basal nitric oxide synthesis in essential hypertension. *Lancet.* 1997;349(9055):837-842. [CrossRef]
11. Gamil S, Erdmann J, Abdalrahman IB, Mohamed AO. Association of NOS3 gene polymorphisms with essential hypertension in Sudanese patients: a case control study. *BMC Med Genet.* 2017;18(1):128. [CrossRef]
12. Go AS, Mozaffarian D, Roger VL, et al. Heart disease and stroke statistics--2013 update: a report from the American Heart Association. *Circulation.* 2013;127(1):e6-e245.
13. Abdullaeva GJ, Abdullaev AA, Kurbanov RD. Molecular-genetic aspects of atrial fibrillation. *O'zbekiston Kardiologiyasi*. 2020;55(1):3-11. https://uzcardio.uz/medias/media/other/37/kardiologiya-1-2020.pdf
14. Laurent S, Boutouyrie P. Recent advances in arterial stiffness and wave reflection in human hypertension. *Hypertens.* 2007;49(6):1202-1206. [CrossRef]
15. Sahn DJ, DeMaria A, Kisslo J, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation.* 1978;58(6):1072-1083. [CrossRef]
16. Devereux RB, Reichek N. Echocardiographic determination of left ventricular mass in man. Anatomic validation of the method. *Circulation.* 1977;55(4):613-618. [CrossRef]
17. Mancia G, Fagard R, Narkiewicz K, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens.* 2013;31(7):1281-1357. [CrossRef]
18. Sharipov RA. Arterial hypertension and diabetes mellitus. *Russian Journal of Cardiology.* 2008;(3):71-75.
19. Hollander W. Role of hypertension in atherosclerosis and cardiovascular disease. *Am J Cardiol.* 1976;38(6):786-800. [CrossRef]
20. Stamler J, Vaccaro O, Neaton JD, Wentworth D. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care.* 1993;16(2):434-444. [CrossRef]
21. Nordestgaard BG, Chapman MJ, Ray K, et al. Lipoprotein(a) as a cardiovascular risk factor: current status. *Eur Heart J.* 2010;31(23):2844-2853. [CrossRef]
22. Linton MF, Yancey PG, Davies SS, et al., editors. Endotext [Internet]. The Role of Lipids and Lipoproteins in Atherosclerosis. South Dartmouth (MA): MDText.com, Inc.; 2000.
23. Klag MJ, Whelton PK, Randall BL, et al. Blood pressure and end-stage renal disease in men. *N Engl J Med.* 1996;334(1):13-18. [CrossRef]
24. Locatelli F, Marcelli D, Comelli M, et al. Proteinuria and blood pressure as causal components of progression to end-stage renal failure. Northern Italian Cooperative Study Group. *Nephrol Dial Transplant.* 1996;11(3):461-467. [CrossRef]
25. Remuzzi G, Benigni A, Remuzzi A. Mechanisms of progression and regression of renal lesions of chronic nephropathies and diabetes. *J Clin Invest.* 2006;116(2):288-296. [CrossRef]
26. Makolkin V, Podzolkov V, Branko V. *Microcirculation in Cardiology*. Moscow: Vizart; 2004:135.
27. Kim HK, Lee H, Kwon JT, Kim HJ. A polymorphism in AGT and AGTR1 gene is associated with lead-related high blood pressure. *J Renin Angiotensin Aldosterone Syst.* 2015;16(4):712-719. [CrossRef]

28. rs4762. SNPedia. Available at: https://www.snpedia.com/index.php/Rs4762 Accessed May 22, 2023.
29. Barouch LA, Harrison RW, Skaf MW, et al. Nitric oxide regulates the heart by spatial confinement of nitric oxide synthase isoforms. *Nature*. 2002;416(6878):337-339. [CrossRef]
30. Ritchie RH, Schiebinger RJ, LaPointe MC, Marsh JD. Angiotensin II-induced hypertrophy of adult rat cardiomyocytes is blocked by nitric oxide. *Am J Physiol.* 1998;275(4):H1370-H1374. [CrossRef]
31. Sládek T, Gerová M, Znojil V, Devát L. Morphometric characteristics of cardiac hypertrophy induced by long-term inhibition of NO synthase. *Physiol Res*. 1996;45(4):335-338.
32. Filip C, Cirstoveanu C, Bizubac M, et al. Pulse Wave Velocity as a Marker of Vascular Dysfunction and Its Correlation with Cardiac Disease in Children with End-Stage Renal Disease (ESRD). *Diagnostics (Basel)*. 2021;12(1):71. [CrossRef]
33. Huber M, Völler H, Jakob S, Reibis R, et al. Role of the angiotensin II type 2 receptor gene (+1675G/A) polymorphism on left ventricular hypertrophy and geometry in treated hypertensive patients. *J Hypertens*. 2010;28(6):1221-1229. [CrossRef]
34. Sethi AA, Nordestgaard BG, Tybjaerg-Hansen A. Angiotensinogen gene polymorphism, plasma angiotensinogen, and risk of hypertension and ischemic heart disease: a meta-analysis. *Arterioscler Thromb Vasc Biol.* 2003;23(7):1269-1275. [CrossRef]
35. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med.* 1998;15(7):539-553. [CrossRef]