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

Özbek Popülasyonunda Renin-Anjiyotensin-Aldosteron Sisteminde Tek Nükleotid Polimorfizmlerin 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 ≤ 0.01; Odds Ratio (OR) = 2.91). The NOS3 −786 T>C variant correlated with left ventricular hypertrophy (P ≤ 0.05; OR = 0.35) and increased pulse wave velocity (P ≤ 0.01; OR = 0.21). The correlations of the AGTR2 1675 G>A variant with left ventricular hypertrophy (P ≤ 0.01; OR = 1.59) and increased pulse wave velocity (P ≤ 0.01; OR = 0.33) were identified. The AGT 704 T>C variant showed a significant association with increased pulse wave velocity (P ≤ 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 araştırmada, Özbek popülasyonunda dokuz polimorfik vartant (rs4961, rs699, rs4762, rs5186, rs1403543, rs1799998, rs5443, rs2070744, rs1799983) ile hipertansiyon oluşumu ve klinik belirtileri arasındaki ilişkii belirlemeye amaçlandktardır.


Bulgular: Polimorfik vartantları genetik analizi AGT 521 C>T vartantının arteriyel hipertansiyon ile anlamlı bir iliştir olduğunu gösterdi (P ≤ 0.01; Odds Orani (OR) = 2.91). NOS3 −786 T>C vartantının sol ventrikül hipertrofisi (P ≤ 0.05; OR = 0.35) ve artmış nabzda hızı (P ≤ 0.01; OR = 0.21) ile ilişkii olduğu bulundu. AGTR2 1675 G>A vartantının sol ventrikül hipertrofisi (P ≤ 0.01; OR = 1.59) ve artmış nabzda hızı (P ≤ 0.01; OR = 0.33) ile korelasyonu tespit edildi. AGT 704 T>C vartantı artmış nabzda hız ile anlamlı bir iliştir göstermektediy (P ≤ 0.05; OR = 2.73).

Sonuç: Çalışılan dokuz polimorfik vartanttaki dördü Özbek popülasyonunda hipertansiyonun klinik belirtiile ilişkilendirilmiştir. Bu vartantlar, Özbek popülasyonunda kardiyovasküler hastalıklar ve hipertansiyon gelişme risklerini belirlemek için genetik biyobelirteç olarak kullanılabilir.

Anatlar Kelimeler: Hipertansiyon, tek nükleotid polimorfizmi, nabzda hızı

ORIGINAL ARTICLE

KLİNİK ÇALIŞMA

Darya Vladimirovna Zakirova1,2
Alisher Abdullaev1,2
Guza Abduulaaev2
Zaringiz Mashkurova2
Elina Aguryanova2
Fozilakhon Omonov2
Sevara Bekmetova2

1Center for Advanced Technologies, Tashkent, Uzbekistan
2Republican 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


DOI:10.5543/tkda.2023.67866

Available online at archivestsc.com. Content of this journal is licensed under a Creative Commons Attribution – NonCommercial-NoDerivatives 4.0 International License.
Cardiovascular diseases (CVD) are among the main causes of morbidity and mortality worldwide, 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. 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.1,2

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.

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...
The formula by Devereux B.R. and co-author. Left Ventricular end-diastole (PWd). Left ventricular mass was calculated using the formula: $LVM = 1.04 \times [(LVEDD+IVSd+PWd)^3-LVEDD^3]-13.6 (g)$. Hemodynamics were studied: left ventricular end-diastolic hemodynamics were studied: left ventricular end-diastolic pressure (LVEDP) and left ventricular wall thickness: interventricular septal dimensions (LVEDD) and left ventricular end-systolic dimensions (LVESD).

**Echocardiographic Assessment**
The echocardiographic assessment was performed using the ultrasonic machine EnVisorC (PHILIPS, Amsterdam, Netherlands) in accordance with the recommendations of the American Society of Echocardiography using M- and B-modes. In M-mode, measurements were obtained through the parasternal axis view in accordance with the recommendations of the Penn Convention method. 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. Left Ventricular Mass (LVM) = $1.04 \times [(LVEDD+IVSd+PWd)^3-LVEDD^3]-13.6 (g)$. 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/m² for males and > 95 g/m² for females.

**Genotyping**
Polyorphic 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 (rs699), AGT C521T (rs4762), AGTR1 A1166C (rs5186), AGTR2 C1675A (rs1403543), CYP11B2 C(-344)T (rs1799998), GNB3 C825T (rs5443), NOS3 T (-786)C (rs2070744), NOS3 G894T (rs1799883).

**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 level was slightly higher among the cases (Table 1). HDL levels were significantly lower among the cases than in the control group.

<table>
<thead>
<tr>
<th>Clinical Parameters</th>
<th>HTN Patients (median, QL, QU)</th>
<th>Healthy (median, QL, QU)</th>
<th>Mann-Whitney U</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>31 (28, 34.75)</td>
<td>27.6 (25, 29)</td>
<td>0.00001</td>
</tr>
<tr>
<td>Blood Glucose (mmol/L)</td>
<td>5 (5, 6)</td>
<td>5.2 (4.975, 5.45)</td>
<td>0.00001</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>147 (110, 211)</td>
<td>100 (72.25, 144.25)</td>
<td>0.0001</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>195 (164.25, 224.5)</td>
<td>184 (170, 219.5)</td>
<td>0.76418</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>43 (37, 51)</td>
<td>50.5 (41, 65)</td>
<td>0.00194</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>109 (70.5, 135.25)</td>
<td>103 (92.5, 140.5)</td>
<td>0.63836</td>
</tr>
<tr>
<td>BUN (mmol/L)</td>
<td>6 (5, 7.25)</td>
<td>5.2 (4, 6)</td>
<td>0.01208</td>
</tr>
<tr>
<td>Creatinine (mmol/L)</td>
<td>87 (71, 100.75)</td>
<td>73.5 (64.5, 86.75)</td>
<td>0.00466</td>
</tr>
<tr>
<td>PWV (m/s)</td>
<td>10 (8.9, 12.8)</td>
<td>7.9 (6.75, 8.9)</td>
<td>0.00128</td>
</tr>
<tr>
<td>LVMI (g/m²)</td>
<td>125 (105, 161.25)</td>
<td>70.5 (63.5, 78)</td>
<td>0.00001</td>
</tr>
<tr>
<td>Microalbuminuria (mg/mL)</td>
<td>16 (6.75, 33)</td>
<td>21 (11, 42.5)</td>
<td>0.22628</td>
</tr>
</tbody>
</table>

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; QC, Lower Quartile (25%); QU, Upper Quartile (75%); TC, Total Cholesterol; TG, Triglyceride.
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 corresponds 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>
<thead>
<tr>
<th>Table 2. Genotype and Allele Frequencies in Two Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>ADD1</td>
</tr>
<tr>
<td>AGT</td>
</tr>
<tr>
<td>AGT</td>
</tr>
<tr>
<td>AGTR1</td>
</tr>
<tr>
<td>AGTR2</td>
</tr>
<tr>
<td>CYP11B2</td>
</tr>
<tr>
<td>GNB</td>
</tr>
<tr>
<td>NOS3</td>
</tr>
<tr>
<td>NOS3</td>
</tr>
</tbody>
</table>
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. 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 ($P < 0.001$). According to the literature, the level of serum oxidative stress is a predictor of mortality in the context of cardiovascular disease. 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.

Our results turned out to be similar to those of German 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 ($P < 0.01$). Notably, the presence of the A allele has a protective effect in both the case of LVH and increased 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.

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 ($>10 \text{ m/s}$) ($P < 0.01$). 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 ($P = 0.002$) 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)
homoyzgous variant of the allele is associated with increased levels of AGT in plasma and high blood pressure.\textsuperscript{24,35} 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.


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


