Research Article / Araştırma Makalesi

ARHGAP42 rs604723 Gene Polymorphism Is Associated With Pulse Wave Speed In Hypertension Diagnosed Subjects

ARHGAP42 rs604723 Gen Polimorfizmi Hipertansiyon Tanısı Konulan Kişilerde Nabız Dalga Hızı ile İlişkilidir

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Abstract

Background: Hypertension is a systemic disease characterized by high blood pressure and an important threat for the population, as it is common and can cause serious complications. Genetic and environmental factors are involved in its development like the recently defined genetic risk factor *ARHGAP42* that encodes the Rho GTPase activating protein 42. In this study, the intronic rs604723 (C/T) gene polymorphism of *ARHGAP42* was investigated in relation to arterial stiffness.
Materials and Methods: Peripheral blood samples, taken from 63 study group subjects with hypertension and 100 healthy subjects as control, were analyzed for the presence of the *ARHGAP42* rs604723 gene polymorphism by a real-time PCR method following DNA isolation. Demographic data of the study group subjects were recorded and blood pressure, ambulatory blood pressure and arterial stiffness values were measured.
Results: The heterozygous polymorphic CT (~2-fold) and homozygous polymorphic TT (~1.6-fold) genotypes were found to be higher in study group subjects when compared to the control group subjects, whereby the increase of the CT genotype was statistically significant ($p = 0.002$). Similarly, the frequency of the polymorphic T allele was found to be higher (~1.9-fold) and statistically significant ($p = 0.003$) in the study group subjects. In addition, the heterozygous polymorphic CT and homozygous polymorphic TT genotypes were determined to be associated with carotid-femoral pulse velocity, which is a measure of arterial stiffness ($p = 0.025$).
Conclusions: In this study, the *ARHGAP42* rs604723 (C/T) gene polymorphism was found to be associated with pulse wave speed in subjects with hypertension. It will be of interest, to investigate its association with any specific drug or drugs commonly used in anti-hypertensive therapy. Thus, it would be possible to select the appropriate drug or drugs according to the hypertensive subjects' genotype to carry out personalized medicine in future.

Key Words: Hypertension, blood pressure, arterial stiffness, *ARHGAP42*, rs604723

Öz

Amaç: Hipertansiyon, yüksek tansiyonla karakterize sistemik bir hastalık olup, yaygın olması ve ciddi komplikasyonlara neden olabilmesi nedeniyle toplum için önemli bir tehdit oluşturmaktadır. Gelişiminde, Rho GTPase aktive edici protein 42'yi kodlayan ve yakın zamanda tanımlanan genetik risk faktörü *ARHGAP42* gibi genetik ve çevresel faktörler rol oynamaktadır. Çalışmada *ARHGAP42*'nin intronik rs604723 (C/T) gen polimorfizminin arteriyel sertlik ile ilişkisi araştırıldı.
Materyal ve Metod: Çalışma grubundaki 63 hipertansiyonlu birey ve 100 sağlıklı kontrol grubundan alınan periferik kan örnekleri, DNA izolasyonunun ardından real time PCR yöntemi ile *ARHGAP42* rs604723 gen polimorfizmi varlığı açısından analiz edildi. Çalışma grubundaki kişilerin demografik verileri kaydedilerek kan basıncı, ambulatuvar kan basıncı ve arteriyel sertlik değerleri ölçüldü.
Bulgular: Heterozigot polimorfik CT (~2-kat) ve homozigot polimorfik TT (~1.6-kat) genotiplerinin çalışma grubundaki bireylerde kontrol grubuna göre daha yüksek olduğu, CT genotipindeki artışın istatistiksel olarak anlamlı olduğu görüldü ($p = 0.002$). Benzer şekilde çalışma grubundaki bireylerin polimorfik T alelinin sıklığı daha yüksek (~1.9-kat) ve istatistiksel olarak anlamlı bulundu ($p=0.003$). Ayrıca heterozigot polimorfik CT ve homozigot polimorfik TT genotiplerinin arteriyel sertliğin bir ölçüsü olan karotis-femoral nabız hızı ile ilişkili olduğu belirlendi ($p = 0.025$).
Sonuç: Bu çalışmada *ARHGAP42* rs604723 (C/T) gen polimorfizminin hipertansiyonlu bireylerde nabız dalga hızı ile ilişkili olduğu belirlendi. Anti-hipertansif tedavide yaygın olarak kullanılan herhangi bir spesifik ilaç veya ilaçlarla ilişkisinin araştırılması ilgi çekici olacaktır. Böylece gelecekte kişiselleştirilmiş tıp uygulamaları için hipertansif bireylerin genotipine göre uygun ilaç veya ilaçların seçilmesi mümkün olabilecektir.

Anahtar Kelimeler: Hipertansiyon, kan basıncı, arteriyel sertlik, *ARHGAP42*, rs604723

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Received / Geliş tarihi: 23.01.2024

Accepted / Kabul tarihi: 13.06.2024

DOI: 10.35440/hutfd.1424247

Introduction

Hypertension is a life-shortening disease of women and men as it has a vast impact on the development of serious complications like heart attack, stroke, congestive heart failure and renal failure. Since hypertension is a multifactorial disease many environmental, genetic, vascular, neuroendocrine and demographic risk factors are found to be involved in its progression. One recently discovered genetic risk factor is a common genetic variation in the Rho GTPase activating protein 42 encoding *ARHGAP42* gene mainly leading to alterations in blood pressure (BP) (1). *ARHGAP42* is selectively expressed in smooth muscle cells (SMCs) and controls BP by regulating the vascular tone (2,3). The BP-associated genetic variation of *ARHGAP42* (rs604723) results from a C to T transition in its first intronic sequence, which at the same time constitutes its regulatory element, and exerts SMC-selective activity. The presence of the minor T allele enhances the activity of the regulatory element by creating a low-affinity binding site for serum response factor (SRF), a transcription factor known to be critical for SMC-specific gene expression. Deletion of this regulatory element in human bronchial SMCs resulted in significantly decreased *ARHGAP42* mRNA expression. On the other hand, cell stretch, hypertension and RhoA-dependent agonists were shown to upregulate *ARHGAP42* expression in vascular SMCs, suggesting that expression of this particular GTPase might act as a negative feedback mechanism to limit excessive vessel constriction. Increased susceptibility of *ARHGAP42*-deficient mice to deoxycorticosterone acetate-salt-mediated hypertension supported this point of view (4).

Arterial stiffness describes the gradual loss of elastic fibres in arteries and the accumulation of stiffer collagen fibres, that occurs as a consequence of biological aging and arteriosclerosis. Since it leads to the development of diseases such as hypertension, chronic kidney disease and stroke it composes another important risk factor for human health (5,6). It is believed that the non-invasive measurement of arterial stiffness is a potentially valuable tool in the detection of early vascular changes that precede hypertension, which could serve to reinforce early life style changes to prevent the development of cardiovascular events and help to direct the most appropriate anti-hypertensive therapy (7).

In this study, we investigated the well-established hypertension risk factor *ARHGAP42* rs604723 gene polymorphism in relation to arterial stiffness.

Materials and Methods

Subjects

Ethical Approval: This study protocol was reviewed and approved by Ege University Faculty of Medicine Clinical Research Ethics, approval number 19-7T/66, date: 31/07/2019

Subjects who applied to Ege University Faculty of Medicine Nephrology and Cardiology outpatient clinic between the years 2018 and 2019 with essential hypertension were included into this study. Exclusion criteria for subjects were secondary causes of hypertension (endocrine, cardiovascular, nephrological and drug-related) and diabetes. Power analysis and sample size estimation were calculated with the GPower 3.1 software; hereunder, for a power of 0.8 the sample size was 63 individuals per group (Table 2). Therefore, our study group consisted of 20 men and 43 women (n = 63), while the control group consisted of 47 healthy men and 53 women (n = 100). Inclusion criteria for the study group subjects were diagnosis of hypertension, systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg or the use of anti-hypertensive medication. Exclusion criteria, on the other side, were coronary heart disease, heart valve disease, cardiomyopathy, kidney or endocrine disorders which are common causes of secondary hypertension. Healthy control group subjects were matched to the study group subjects by gender and age. Hardy-Weinberg equilibrium (HWE) of the control group was not significant.

Blood Pressure Measurements

Demographic data of the study group subjects were recorded and blood pressure, ambulatory blood pressure and arterial stiffness values were measured. Blood pressure was measured by specialised medical personnel after the participants had rested for 10 minutes in a quiet environment. Blood pressure measurements were repeated 3-times before the average of systolic and diastolic blood pressures was recorded. Systolic blood pressure of <140 mmHg and diastolic blood pressure of <90 mmHg were accepted as normal blood pressure, while ≥140 mmHg systolic blood pressure and/or ≥90 mmHg diastolic blood pressure values were accepted as hypertension. Ambulatory blood pressure (ABP) was recorded with the Spacelabs Medical DPI; V2.0.6 device every 30 minutes between 08:00 and 23:00 and every hour between 23:00 and 08:00. After measurements were taken, day and night systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (OCP) and heart rate values for 24 hours were obtained.

Arterial Stiffness Measurements

Pulse wave analysis and PWV measurements were done following the methodology described in our previous study (8). All measurements were made in the supine position, heart rate was monitored at the time of measurement, and only portions with stable sinus rhythm were analyzed. Carotid femoral pulse wave velocity was also measured by a cardiologist with Doppler echocardiography. Vivid E9, GE W healthcare Doppler echocardiography with a linear array probe and electrocardiography synchronized was used for measurements. Two examination areas were used: 1. the common right carotid and 2. the common right femoral artery. Pulse wave Doppler samples were performed at the

two relating arteries. The pulsed wave Doppler sample volume was set to 5.07 mm the low velocity filter reduced in order to get the beginning of the wave adjacent with the base line. Electrocardiography gating mode was a prerequisite for assessing the time travel of the pulse wave. Time travel was measured as the time interval between the peak R to the onset of the pulse wave in the common carotid artery and the peak R to the onset of the pulse wave of the common femoral artery. The difference of these time intervals represented the time that it took for the pulse wave to travel from the common carotid artery to the common femoral artery. A sweep speed of 100 m/sec was used to record the mean of six Doppler waveforms. Using a flexible measuring tape, the distance between two Doppler recording spots was determined over the body surface. The distance from the suprasternal notch to the umbilicus was pieced in the extension from the umbilicus to the right groin. An experienced echocardiographer conducted both the Doppler echocardiography and the distance measures.

Bioelectrical impedance analysis

Bioelectrical impedance analysis was performed at four frequencies (5, 50, 100 and 200 kHz) with a Quadscan 4000 (BodyStat, UK) multifrequency device on the right calf of the study group in the supine position after draining the dialysis solutions. BIA was used to measure extracellular water (ECW), intracellular water (ICW), TBW, and total body fat (9).

Carotid artery intima-media thickness measurement

The common carotid arteries were the subject of ultrasonographic examinations using high-resolution color Doppler ultrasound (Sonoline Antares, Siemens, Germany, equipped with VFX 13–5 transducer). The single operator performed all procedures on both sides of two longitudinal images of each common carotid artery. The mean CA-IMT was calculated using the average of four values obtained from measurements of the carotid bulb and distal common carotid artery on each side. The intraobserver coefficient of variation was 2.5%.

Polymorphism Analysis

DNA was isolated from peripheral blood leucocytes of all subjects by using the High Pure PCR Template Preparation Kit (Roche, Cat. #11796828001). DNA purity and concentration were measured with the NanoDrop ND-1000 instrument. For the determination of the cytosine to thymine transition (C→T or C/T) of the rs604723 gene polymorphism located in the first intron of the *ARHGAP42* gene, specific primers and a probe mixture (LightSNiP Assay, TIB MOLBIOL) were used together with the LightCycler® DNA Master HybProbe (Roche, Cat # 12015102001) kit and studied on the real-time PCR LightCycler 480 Instrument II (Roche).

Melting curve analysis was used to determine the alleles which was 51 ± 2°C for the C allele and 58 ± 2°C for T allele.

Statistical Analysis

Statistical analysis was performed by using IBM SPSS 21.0. All variables were checked for normality using probability plots and Kolmogorov-Smirnov/Shapiro-Wilk tests. Results were expressed as means - standard deviations or median - interquartile range (IQR) for normally or non-normally distributed variables. The differences in genotype distribution were assessed by Chi-square test. Differences between means were tested by t-test, ANOVA, or Kruskal-Wallis test, where appropriate. Multiple comparisons were performed using Bonferroni or Dunnett's T3 post hoc tests. The level of significance was accepted as $p < 0.05$.

Results

Study Group Characteristics

The study group subjects were older than the control group subjects with mean ages of 54.16 ± 11.841 and 51.62 ± 7.326, respectively ($p < 0.01$). Rates of anti-hypertensive drug usage by the study group subjects were; angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) 58 %, diuretics 23 %, calcium channel blockers (CCBs) 19 % and beta-blockers 12. 5 %. Whereas, number of subjects and rates of anti-hypertensive drugs used were as follows; subjects using one drug 35.4 %, two drugs 14.6 %, three drugs 10.4 %, and four drugs 4.2 %. Eventually, the rate of study group subject that did not use any anti-hypertensive drug was 35.4 % (data not shown). Study group characteristics are given in detail in Table 1.

ARHGAP42 rs604723 Genotype Distribution and Allele Frequency

Genotype distribution and allele frequency of the *ARHGAP42* rs604723 (C/T) gene polymorphism are given in Table 2. According to this, 73 subjects in the control group carried the homozygous wild type CC (73 %), 22 subjects the heterozygous polymorphic CT (22 %) and 5 subjects the homozygous polymorphic TT (5 %) genotype. Whereas, in the study group 30 subjects carried the homozygous wild type CC (47.6 %), 28 subjects the heterozygous polymorphic CT (44.4 %), and 5 subjects the homozygous polymorphic TT (8 %) genotype. A statistically significant increase in the heterozygous polymorphic CT genotype was observed in the study group compared to the control group when the two groups were compared (p = 0.002). Similarly, when the allele frequency between the control and study groups was examined, again a statistically significant increase of the polymorphic T allele frequency in the study group could be shown (p = 0.003).

ARHGAP42 rs604723 in Relation with Clinical and Biochemical Parameters

In order to determine the relationship of the *ARHGAP42* rs604723 (C/T) gene polymorphism with clinical and biochemical parameters, statistical analyzes were performed based on the genotypes of the study group subjects (Table 3). According to this, the correlation of the heterozygous polymorphic CT genotype with intracellular fluid volume

(ICW), nutrition and body cell mass (BCMASS) parameters were found to be statistically significant ($p < 0.05$). Genotypes of the subjects were not associated with diabetes (DM), thyroid disease, alcohol and smoking consumption, left ventricular diastolic dysfunction (LVDD) and hyperlipidemia (HLP) ($p > 0.05$; data not shown). Again, no statistically significant difference was found between the genotypes of the subjects that used and did not use anti-hypertensive drugs ($p = 0.132$). However, when the subjects with the heterozygous polymorphic CT and homozygous polymorphic TT genotypes were compared with those with the homozygous wild-type CC genotype, although statistically not significant anti-hypertensive drug usage was found to be increased ($p = 0.057$; OR: 4.05).

Table 1. Hypertension Study Group Characteristics

	Mean ± SD		Median-IQR
Age	54 ± 11		
Sex (F, %)	70		
WHRATIO	0.86 ± 0.08		
BMI	29.3 ± 4.5		
Smoking (%)	28		
Duration of HT (years)	9.6 ± 8.2		
Antihypertensive drug Use (%)		Antihypertensive drug Number (%)	
ACEI/ARB 58		1 drug 35.4	
Diuretics 23		2 drugs 14.6	
CCBs 19		3 drugs 10.4	
BB 12.5		4 drugs 4.2	
SP (mmHg)	131 ± 20		
DP (mmHg)	85 ± 12		
PP (mmHg)	51 ± 15		
AI (%)	34 - 15		
AIHR75 (%)	32 - 13		
ED	314 ± 26		
C_MEANP	103 ± 14		
C_SVI	147 ± 32		
HR	72 ± 14		
PWV (m/s)	7.6 ± 1.3		
Echocardiography and Pulse Wave Analysis			
VKI	29.2 ± 4.9	IVS	9 - 2
LA	34.3 ± 4.6	RV	25 - 5
LVEDD	44.8 ± 5.6	LVEF	60 - 0.00
LVESD	31.11 ± 5.1	LVMI	66 - 26
LVPW	8.60 ± 1.46	SPAP	25 - 0
LVM (GR)	129 ± 40	CCAANTIMT	0.63- 0.14
CCAPOSTIMT	0.69 ± 0.15	C-F PWV(MS)	9.9 - 4.3
Bioimpedance Analysis			
THIRDSPACE	0.75 ± 1.15	BCMASS	28.4- 6.6
		FAT	26.2- 11.5
		LEAN	44.7- 13.1
		WATER	35.5- 7.5
		DRY	10.3- 3.5
		ICW	19.9- 4.6
		NORMECW	15.5- 5.2
		TBW	36.8- 8.2
		NORMNUTR	0.40- 0.03
		IMPIDX	0.78- 0.05

SD: Standard Deviation, IQR: Interquartile Range, BMI: Body Mass Index, BB: Beta-blocker, SP: Systolic Blood Pressure, DP: Diastolic Blood Pressure, PP: Pulse Pressure, ED: Ejection Duration, CMEANP: Central Mean Pressure, C_SVI: Central Subendocardial Viability Index, HR: Heart Rate, NORMICW: Normalized Intracellular Volume, ECW: Extracellular Volume, PWV: Pulse Wave Velocity, WHRATIO: Waist-Hip Ratio, BCMASS: Body Cell Mass, AI: Augmentation Index, AIHR75: Augmentation index adjusted to heart rate of 75, ICW: Intracellular Fluid Volume, NORMECW: Normalized Extracellular Volume, TBW: Total Body Volume, NORMNUTR: Normalized Nutrition, IMPIDX: Impedance Index, VKI: vena cava inferior diameter, LA: Left Atrium, LVEDD: Left Ventricular End Diastolic Diameter, LVESD: Left Ventricular End Systolic Diameter, LVPW: Left Ventricular Posterior Wall Thickness, LVM: Left Ventricular Mass, CCAPOSTIMT: Common Carotid Artery Posterior Intima Media Thickness, IVS; Interventricular Septal Wall Thickness, RV: Right Ventricle, LVEF: Left Ventricular Ejection Fraction, LVMI: Left Ventricular Mass Index, SPAP: Systolic Pulmonary Artery Pressure, CCAANTIMT: Common Carotid Artery Anterior Intima Media Thickness, C-F PWV: Carotid-Femoral Pulse Wave Velocity.

Table 2. Genotype Distribution and Allele Frequency of the rs604723 Polymorphism

	Genotype/ Haplotype	Control *n* = 100 (%)	HWE	HT *n* = 63 (%)	OR	95% CI	*p*
rs604723	CC	73 (73.0)	0.069451	30 (47.6)	1		
	CT	22 (22.0)		28 (44.4)	3.097	1.535-6.248	**0.002**
	TT	5 (5.0)		5 (8.0)	2.433	0.656-9.022	0.184
	C	168 (84.0)		88 (69.8)	2.267	1.326 - 3.876	**0.003**
	T	32 (16.0)		38 (30.2)			

Power (1-β err prob) of the study was 0.9512656 with critical χ^2:10.8306014, α:err prob: 0.004448, noncentrality parameter λ: 23.2523498. HWE: Hardy-Weinberg Equilibrium, HT: Hypertension, OR: Odds ratio, CI: Confidence interval.

Table 3. rs604723 in Relation with Clinical and Biochemical Parameters in Hypertension Study Group

rs604723	**Genotype**			*p*
	CC	CT	TT	*value*
BMI	29.862 ± 3.27	28.55 ± 5.21	32.14 ± 6.67	0.340
SP	130.09 ± 21.39	132.04 ± 20.12	137.67 ± 12.22	0.820
DP	84.04 ± 11.41	87.17 ± 14.85	89.33 ± 8.08	0.643
P_MEANP	101.22 ± 12.45	104.46 ± 17.06	109.00 ± 2.64	0.591
C_SVI	142.48 ± 32.01	151.96 ± 34.49	158.00 ± 5.57	0.528
HR	75.26 ± 17.47	70.54 ± 12.65	69.33 ± 4.72	0.518
AI (%)	26.08 ± 15.93	32.25 ± 10.42	38.00 ± 6.08	0.159
AIHR75 (%)	27.23 ± 9.72	30.08 ± 10.40	35.33 ± 5.50	0.341
C_SP	121.39 ± 19.56	124.75 ± 20.36	131.67 ± 10.78	0.648
C_DP	85.26 ± 11.81	88.29 ± 15.09	90.67 ± 7.64	0.661
C_MEANP	101.22 ± 12.45	104.46 ± 17.06	109.00 ± 2.64	0.591
PWV	7.59 ± 0.94	7.71 ± 1.62	8.33 ± 1.17	0.664
FAT	27.22 ± 7.83	27.79 ± 8.35	32.23 ± 16.73	0.656
LEAN	52.92 ± 11.41	46.29 ± 8.92	44.43 ± 6.05	0.087
WATER	39.85 ± 7.11	35.71 ± 5.23	35.97 ± 3.13	0.095
DRY	13.063 ± 4.80	10.57 ± 3.99	8.47 ± 3.17	0.097
WHRATIO	0.87 ± 0.08	0.8473 ± 0.09	0.85 ± 0.04	0.562
ICW	22.86 ± 4.17	19.80 ± 3.79	20.80 ± 2.14	**0.049**
NORMICW	25.32 ± 4.35	22.38 ± 4.95	23.90 ± 3.58	0.141
ECW	17.70 ± 2.56	16.64 ± 2.09	16.47 ± 1.20	0.303
NORMECW	17.94 ± 4.13	15.18 ± 3.86	16.67 ± 2.39	0.092
TBW	40.56 ± 6.48	36.43 ± 5.83	37.27 ± 3.09	0.101
NORMTBW	43.27 ± 8.44	37.55 ± 8.77	40.57 ± 5.76	0.113
NUTRITION	0.44 ± 0.02	0.46 ± 0.02	0.44 ± 0.02	**0.005**
NORMNUTR	0.41 ± 0.01	0.40 ± 0.01	0.41 ± 0.02	0.100
BCMASS	32.65 ± 5.95	28.28 ± 5.42	29.70 ± 3.03	**0.049**
IMPIDX	0.75 ± 0.07	0.79 ± 0.03	0.78 ± 0.03	0.077

BMI: Body Mass Index, SP: Systolic Blood Pressure, DP: Diastolic Blood Pressure, C_SVI: Central Subendocardial Viability Index, HR: Heart Rate, AI: Augmentation Index, AIHR75: Augmentation index adjusted to heart rate of 75, CMEANP: Central Mean Pressure, PWV: Pulse Wave Velocity, WHRATIO: Waist-Hip Ratio, ICW: Intracellular Volume, NORMICW: Normalized Intracellular Volume, ECW: Extracellular Volume, NORMECW: Normalized Extracellular Volume, TBW: Total Body Volume, NORMTBW: Normalized Total Body Volume, NORMNUTR: Normalized Nutrition, BCMASS: Body Cell Mass, IMPIDX: Impedance Index.

ARHGAP42 rs604723 in Relation with Cardiovascular Parameters

In order to determine the relationship of the *ARHGAP42* rs604723 (C/T) gene polymorphism with cardiovascular parameters, statistical analyzes were performed based on the genotypes of the study group subjects (Table 4). When subjects with the heterozygous polymorphic CT and homozygous polymorphic TT genotypes were compared with those with the homozygous wild-type CC genotype the carotid-femoral pulse wave velocity (cfPWV) value, which is used to assess arterial stiffness and measured by echocardiography was found to be statistical significantly increased (p = 0.025).

Table 4. rs604723 in Relation with Cardiovascular Parameters Hypertension Study Group

rs604723	Genotype			*p value*
	CC	CT	TT	
VKI	29.79 ± 3.72	27.96 ± 5.01	34.60 ± 7.21	0.124
SYSTOLIC	128.18 ± 18.86	125.17 ± 16.19	144.00 ± 5.65	0.349
DIASTOLIC	81.18 ± 11.04	75.33 ± 12.88	83.50 ± 4.95	0.300
HR	77.71 ± 14.82	72.39 ± 14.13	72.50 ± 3.536	0.536
HTD (years)	9.941 ± 9.73	7.083 ± 8.93	7.000 ± 1.41	0.644
LA	34.00 ± 4.11	35.17 ± 5.33	38.00 ± 9.89	0.517
LVEDD	43.94 ± 3.68	43.39 ± 6.80	47.50 ± 0.71	0.600
LVESD	30.71 ± 3.67	28.94 ± 5.33	31.00 ± 9.89	0.535
IVS	10.06 ± 2.51	9.06 ± 1.55	8.50 ± 0.71	0.287
LVPW	8.53 ± 1.32	8.89 ± 1.41	10.00 ± 0.00	0.323
RV	23.41 ± 5.85	24.39 ± 2.95	25.00 ± 0.00	0.774
LVEF	60.00 ± 0.00	59.17 ± 2.57	60.00 ± 0.00	0.389
LVM (GR)	127.53 ± 36.57	128.00 ± 43.76	150.50 ± 3.53	0.737
LVMI	64.94 ± 15.75	71.72 ± 23.85	81.00 ± 5.65	0.428
SPAP	26.47 ± 4.24	28.72 ± 9.77	32.00 ± 8.48	0.509
SOLCCAPOSTIMT	0.73 ± 0.15	0.73 ± 0.15	0.60 ± 0.02	0.464
SOLCCAANTIMT	0.66 ± 0.14	0.66 ± 0.11	0.67 ± 0.03	0.984
CFPWV (MS)	9.45 ± 2.09	11.05 ± 2.52	14.30 ± 6.29	0.025

VKI: vena cava inferior diameter, HR: Heart Rate, HTD: hypertension duration, LA: Left Atrium, LVEDD: Left Ventricular End Diastolic Diameter, LVESD: Left Ventricular End Systolic Diameter, IVS: Interventricular Septal Wall Thickness, LVPW: Left Ventricular Posterior Wall Thickness, RV: Right Ventricle, LVEF: Left Ventricular Ejection Fraction, LVMASS: Left Ventricular Mass, LVMI: Left Ventricular Mass Index, SPAP: Systolic Pulmonary Artery Pressure, CCAPOSTIMT: Common Carotid Artery Posterior Intima Media Thickness, CCAANTIMT: Common Carotid Artery Anterior Intima Media Thickness, CFPWV: Carotid-Femoral Pulse Wave Velocity.

Discussion

The Rho GTPase activating protein 42 (*ARHGAP42*) gene, which is located at chromosome 11q22.1, encodes a member of the Rho GTPase activating protein (RhoGAP) family. *ARHGAP42* was first identified as a protein synthesized in SMCs with a function in controlling BP by regulating the vascular tone (9). It has been demonstrated that *ARHGAP42* expression functions as a pressure-sensitive rheostat to control vascular tone by reducing Ca^{2+}-sensitivity and limiting the expression of specific contractile proteins in SMCs supporting this function (3,10,11). The fact that four single nucleotide polymorphisms (rs604723, rs633185, rs607562, rs667575) in its first intronic sequence were found to be associated with hypertension, underpinned its potential therapeutic value in hypertension control (4,12,13). In a detailed functional study, the DNase-hypersensitivity region 2 (DHS2) in its first intronic sequence has been shown to regulate its expression and that it is actually the rs604723 (C/T) gene polymorphism located into this DHS2 that is associated with BP. According to this, the presence of the minor T allele creates a consensus binding element for SRF, a transcription factor known to be critical for SMC-specific gene expression, whereas the major C allele inhibits SRF binding to this sequence (4,14).

Large artery walls, especially the aorta, lose elasticity over time leading to arterial stiffness. Increased arterial stiffness, on the other hand, is closely linked to increased risk of developing hypertension, chronic kidney disease and stroke (6,15). Nevertheless, by measuring the structural and functional properties of blood vessels the preclinical stage of vascular disorders can be assessed. Recent research has shown that arterial stiffening precedes the development of high BP and can be used to estimate future cardiovascular events. Since several experimental models of various conditions proved that arterial stiffness is reversible, this knowledge can be used to prevent the development of hypertension or other diseases. Therefore, understanding the biological mechanisms of arterial stiffening and investigating potential therapeutic interventions to modulate arterial stiffness is essential and requires reliable parameters, devices and standardized arterial stiffness measurement protocols (5). Since, the minor T allele of the *ARHGAP42* rs604723 gene polymorphism was shown to be associated with reduced diastolic BP in subjects with untreated borderline hypertension, we wanted to investigate if this allele is also associated with arterial stiffness in subjects diagnosed with hypertension.

When we analysed the genotype distribution of the *ARHGAP42* rs604723 gene polymorphism the heterozygous polymorphic CT and homozygous polymorphic TT genotypes were found to be more frequent in the study group subjects with hypertension than in the healthy control group subjects, with increase of the CT genotype being statistically significant. Also, statistically significant the frequency of the polymorphic T allele was found to be increased in the study

group subjects. These results once more confirmed the association of the *ARHGAP42* rs604723 polymorphic T allele with hypertension.

The relationship of hypertension with age is well known and documented, i.e. the risk of developing hypertension disease increases with age (16,17). Similarly, hypertension is also associated with gender; in other words, diastolic hypertension is more common in men of all age ranges, while high systolic blood pressure is more common in women aged > 60 years (18). Since, attention was paid that age and gender distributions of study and control group subjects were balanced, no association was found between these parameters and the *ARHGAP42* rs604723 gene polymorphism in our study. In addition, genotypes of the hypertension study group subjects were not found to be associated with diabetes, thyroid disease, alcohol or cigarette consumption, left ventricular diastolic dysfunction and hyperlipidemia parameters. However, correlation of the heterozygous polymorphic CT genotype with intracellular fluid volume, nutrition and body cell mass parameters was statistically significant. It was also noticed that use of anti-hypertensive drugs was higher and statistically significant in study group subjects with heterozygous polymorphic CT and homozygous polymorphic TT genotypes compared to those with homozygous wild type CC genotypes. Although not statistically significant, study group subjects with the heterozygous polymorphic CT and homozygous polymorphic TT genotypes were found to use more anti-hypertensive drugs which might be associated with increased arterial stiffness. It is argued that different anti-hypertensive drug classes may have different effects on arterial stiffness; e.g. while ACE inhibitors, CCBs, and mineralocorticoid receptor antagonists (MCRAs) are beneficial in reducing arterial stiffness and central BP; some beta-blockers may have the opposite effects while lowering peripheral BP. But, it should be also kept in mind that the majority of studies on beta-blockers have investigated the effects of atenolol and there are insufficient data available regarding the effects of vasodilating beta-blockers like labetalol. While ARBs appear to have a beneficial effect on arterial stiffness, diuretics appear to be neutral, as they do not appear to affect arterial stiffness and central BP beyond their effects on reducing brachial artery pressure (7). In accordance with our findings, when subjects with resistant hypertension, i.e. BP values remaining above the therapeutic goal despite concurrent use of three antihypertensive agents from different classes, were compared to subjects with well controlled hypertension their brachial-ankle pulse wave velocity (baPWV) was found to be increased indicating to elevated vascular stiffness (19). Concisely, the need of too many anti-hypertensive drugs in the control of hypertension is an indicator of BP severity and a risk factor for arterial stiffness in affected subjects.

In our study, systolic and diastolic blood pressure values were not found to be associated with the genotypes of the study group subjects, which is mainly due to the fact that most of them were using anti-hypertensive drugs for their therapy. However, when subjects with the heterozygous polymorphic CT and homozygous polymorphic TT genotypes were compared with those with homozygous wild-type CC genotype, cfPWV an indicator for arterial stiffness measured by echocardiography and SphygmoCor was found to be statistically significant increased. This finding again might explain the reason why subjects carrying those genotypes were described more anti-hypertensive drugs; probably due to greater arterial stiffness and difficulties encountered in blood pressure control.

Drugs that are commonly used in the control and treatment of hypertension and hypertension- related cardiovascular diseases do not exert the same therapeutic effect in all cases, are sometimes insufficient or even cause adverse effects. This is partly due to different hypertension-susceptibility genes involved in the disease outcome of the subjects and other genetic variations with additive effects in the clinical course of the disease or drug response (20). Thus, intensive research for new blood pressure reducing agents and drugs is carried out as well as for novel therapeutic targets for anti-hypertensive therapies that could prove useful for individualizing treatment regimens. Hypertension can sometimes develop as a consequence of either overexpression or downregulation of one risk factor genes. In such cases, gene therapy-based anti-hypertensive drugs could be used to target the responsible gene and reverse this disease-causing condition. In a study, where survivin, a protein inhibiting apoptosis, was downregulated by its dominant-negative mutant in pulmonary arterial hypertension (PAH) rat models lowered pulmonary vascular resistance, right ventricular hypertrophy, and pulmonary arterial medial hypertrophy could be achieved (21). Similarly, antisense oligonucleotides (ASOs) against angiotensin II receptor type 1 (ATR1), angiotensinogen (AGT), ACE or beta 1-adrenergic receptor (ADRB1) genes were also successfully used in the treatment of hypertension in rat models. Administration of ASOs in liposomes even prolonged their therapeutic effects (22). So far, there are no clinical trials investigating candidate drugs able to modulate *ARHGAP42* expression or ASOs that specifically bind and inhibit the minor T allele of the rs604723 gene polymorphism in the treatment of primary hypertension. But, with the development of new small molecular drugs it will likely be possible to regulate enzymes like *ARHGAP42* that could be effectively used in anti-hypertensive therapies (20,23,24).

In summary, we found that the *ARHGAP42* rs604723 (C/T) gene polymorphism is associated with pulse wave speed in subjects diagnosed with hypertension. Since, the number of anti-hypertensive drugs used usually increases in subjects with arterial stiffness, it would be also interesting to investigate the association between specific anti-hypertensive drugs and the different *ARHGAP42* rs604723 gene polymorphism genotypes in a larger cohort. A significant association would be useful in the future to select the appropriate anti-hypertensive drug or drugs according to the subjects' genotype to carry out personalized therapy.

Ethical Approval: This study protocol was reviewed and approved by Ege University Faculty of Medicine Clinical Research Ethics, approval number 19-7T/66, date: 31/07/2019

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Conflict of Interest: *The authors have no conflicts of interest to declare.*

Financial Disclosure: *This study evaluated by the Medical Health Group was approved as project ID 20208 and funded by the Ege University Research Fund.*

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