Iraqi Journal of Biotechnology, 2024, Vol. 23, No. 1, 159-164

Impact of Gene Polymorphisms (Rs5046) and Serum Levels of *AGT* Gene Attribution in Prevalence of Hypertension Patients

[1]Marwah Y. Hameed, [1]Rafid A. Abdulkareem

[1]Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad

Received: June 1, 2023 / Accepted: September 10, 2023/ Published: September 23, 2024

Abstract: Some genes might probably contribute to the regulation of blood pressure due to their previously established effect on cardiovascular or renal function, in addition to physical and environmental factors. The aim of the study detects the relationship between the genetic polymorphism of AGT and the level of AGT in serum and its association with high blood pressure patients. The *AGT* levels in serum have been measured by ELISA kit. In this study, Genomic DNA was extracted by DNA extraction kit from both patients and control group and the measurement of concentration and purity was done by the use of nanodrop. (T-ARMS-PCR) was used to determine the SNPs rs5046 (G/A) in the 5′ near region of *AGT* gene in hypertension patients and healthy controls, by using specific Primers**,** The Results detected that genotype CC (34 (68.0) % *vs*. 50 (100.0) %) was decreased frequency in patients than the control group. In contrast, the CT genotype showed a significant increase (16 (32.0) *vs*. 0 (0.0) %; OR = 48.30; $p<0.001$) in the patient group compared with the control group. While the TT genotype does not appear in any groups of patients and controls. The results of the assessment of AGT plasma levels in *AGT* rs5046 SNP genotypes showed no significant differences according to the rs5046 genotypes in the level of AGT. For CC genotypes, AGT levels were higher in the hypertension patient group than in the control group and significantly ($p<0.001$). It was concluded investigation indicate that CT was a risk factor for hypertension (EF =31.7).

Keywords: Angiotensinogen, serum levels, hypertension, Rs5046, gene polymorphisms.

Corresponding author: (Email: marowabio1985@gmail.com).

Introduction

Blood pressure is the amount of pressure that the circulatory system's blood pumps via the arteries at every moment. Systolic blood pressure, or the top number, is the force of pressure that blood exerts on the artery walls as the heart contract. The diastolic blood pressure (the bottom number), is the pressure on the artery wall when the heart relaxes between beats. (1, 2). Mean Arterial Pressure (MAP) refers to the average blood pressure throughout a single cardiac cycle, whereas Pulse Pressure (PP) refers to the difference in (mmHg) between the systolic and diastolic pressures (3). Given that it typically causes no symptoms, it has been dubbed a silent killer. Since it takes a while for hypertension to be detected, serious health issues like stroke and other cardiovascular disorders might result. The long-term effects of high blood pressure sickness include harm to organs like the brain, heart, kidneys, eyes, and other body parts (4). The 95 per cent of those with hypertension suffer from essential

hypertension, commonly referred to as primary hypertension or idiopathic hypertension. It is often inherited and thought to be brought on by a confluence of genetic and environmental factors.

Essential hypertension becomes increasingly common as people age, and persons who have moderately high blood pressure at an early age are more likely to develop hypertension later in life, causing them significant pain. Hypertension increases the likelihood of a stroke, heart attack, and kidney failure(5).

Some genes might probably contribute to the regulation of blood pressure due to their previously established effect on cardiovascular or renal function, in addition to physical and environmental factors. Due to the candidate gene strategy, it is assumed that a given gene or more probably a set of genes that are involved in a specific function, can contribute to the variation of blood pressure (6). A high risk of hypertension has been linked with angiotensinogen (*AGT*), angiotensin-converting enzyme (*ACE*), and angiotensin II receptor 1 (*AGTR1*) (7). Angiotensinogen is a protein that is produced as a result of the AGT gene.

This protein is a component of the renin-angiotensin system, which controls the body's salt, fluid, and blood pressure levels. Angiotensinogen is converted to angiotensin I in the first phase and Angiotensin I is converted to angiotensin II in a subsequent stage. A genetic variant in the promoter of the AGT gene leads to a change in the level of the AGT protein (2).

Carriers of this variant are salt-sensitive, which implies that compared to non-carriers, they retain more sodium in their blood, Blood volume increases with a high sodium intake, which raises blood pressure. Therefore, those who carry this AGT variation are more likely to acquire hypertension (8). The aim of the study detects the relationship between the genetic polymorphism of *AGT* and the level of AGT in serum and its association with high blood pressure patients.

Materials and methods

This study was done at the University of Baghdad's Institute of Genetic Engineering and Biotechnology for Post-Graduate Studies between November 2020, and March 2021. fifty patients suffering from hypertension who attended the Ibn Al-Bitar Centre for Cardiac Surgery Hospital were included in the study. Their ages ranged from (50 to 78) years. Fifty people have normal blood pressure as a healthy control. with age (30 to 70) years. The questionnaire asks about age, gender, and family history.

Detection of rs 5046 in AGT gene by using tetra arms technique

Genomic DNA was extracted by DNA extraction kit from both patients and control group and the measurement of concentration and purity was done by the use of nanodrop. The Tetra-Amplification-Refractory Mutation System-Polymerase Chain Reaction (T-ARMS-PCR) was used to determine the SNPs r5046 (G/A) in the 5′ near region of AGT gene in hypertension patients and healthy controls, by using specific Primers were designed for this technique according to their sequence in NCBI by free online primer designing tool https://www.ncbi.nlm.nih.govpmc and http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi.

The primer sequence and product are displayed in (Table 1). PCR reactions were performed in 25 µl PCR tubes under sterile conditions. The components were mixed in 0.25 ml Eppendorf tube the reaction mixture

was listed in (Table 2). The optimization of amplification for *AGT* was performed under the following conditions in (Table 3). The PCR products and the ladder marker were resolved by electrophoresis. 20µl of PCR amplified products were loaded on 2% agarose gel (1g agarose/50 ml 1X TBE buffer) and run at 50 volts for 60min. The gel was stained with ethidium bromide solution. Estimation of AGT serum levels was done by using ELISA Kits Mybiosource / U.S.A. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of AGT. Calculate the concentration of AGT in the samples by comparing the OD of the samples to the standard curve.

Statistical analysis of data

The probability was examined by using the independent T-test and ANOVA table (Duncan test). For non-parametric data, Pearson's chi-square test is used to calculate the probability. A Pearson's correlation was used to determine the relationship between the studied parameters. For genotyping and alleles frequencies, the odd ratio, 95% confidence interval and Fisher's exact probability were calculated by WinPepi version 11.65. Online Hardy-Weinberg calculator used for genotyping and alleles frequency calculations.

Table (1): The primers and their sequences used in Tetra arms Technique

No.	SNP	Type	Primer sequence 5′-3′	Size product
1	*AGT* Rs5046	Inner F	GCAAAGGCCTCTAATAGACACTGTGA	A allele: 163
		Inner R	CACTTTTCACTTGCTTGTGTGTTGTC	G allele: 212
		Outer F	GGGAGATGTACCCCCAAGAGG	outer primer:323
		Outer R	ACATCTTTCAATGCCTGCATCCT	

Table (2): The PCR reaction components for amplifying the targeted fragments.

Component	Amount (µl)
DNA Template	3
PCR Master Mix 2X	5
Inner forward primer	1.5
Inner reverse primer	1.5
Outer forward primer	1
Outer revers primer	1
Distell water	12
Total	25

(Table 3): Optimization of PCR conditions for *AGT* gene

No.	Step	Temperature	Time	Cycles
1	Initial -denaturation	95C°	5 min	1
2	Denaturation	95C°	1 min	
3	Annealing	61C°	1 min	45
4	Extension	72C°	1 min	
5	Final Extension	72C°	7 min	1
6	Storage	4 C°	∞	

Results and discussion

The (T-ARMS-PCR) is a simple device that meets the expectations of modern diagnostic laboratories. It is a fast, reliable and cost-effective genetic analysis method for genetic

diseases(12). The present study uses (T-ARMS-PCR) to determine the SNPs r5046 (G/A) in the 5′ near the region of the AGT gene in hypertension patients and healthy controls, by using a specifically designed primer. The T-ARMS-PCR product of the samples was divided into three bands (323bp) representing the product size of two outer primers which is used as a DNA template for the inner PCR (163 bp) as for the allele (T), (212bp) for the allele (C). The Wild-Type genotype (CC) would have only two bands (212 and 323 bp). The hetero-genotype (CT) would have three bands of (323, 212, and 163 bp), and the mutant homo genotype (TT) would have two bands (323and163). The CC, and CT genotypes as shown in (Figures 1,2).

The results in (Table 4) refer to genotype CC (34 (68.0)% vs. 50 (100.0)%) was decreased frequency in patients than the control group. In contrast, the CT genotype showed a significant increase (16 (32.0) vs. 0 (0.0) %; OR = 48.30; p<0.001) in the patient group with hypertension compared with the control group. While the TT genotype does not appear in any groups of patients and controls. The findings of the current investigation indicate that CT was a risk factor for hypertension (EF =31.7). The T allele showed significantly increased frequency in hypertension patients compared to the control group (16 (16.0) % *vs*. 0 (0.0) %; OR= 39.25; p<0.001). According to these results, the T allele seems to be a risk allele. The C allele frequency observed a significantly decreased frequency in patients compared to the control group (84 (84.0) % *vs*100 (100.0) %; OR = 0.03; p<0.001).

There was a substantial difference (p > 0.05) between the observed and anticipated frequencies of these genotypes in hypertension patients, according to Hardy-Weinberg equilibrium (HWE) analysis.

Correlation between *SNP* rs5046 in *the* promoter of *AGT* gene and serum level of AGT

The assessment of AGT plasma levels in AGT rs5046 SNP genotypes showed different results in hypertension patients and controls (Table 5). There were no notable variations in the levels of AGT between the two groups, patients and controls, within any of the two groups. For the CC genotype, the AGT level was considerably (p 0.001) greater in the hypertension patient group than in the control group. CT and TT genotypes were not statistically accounted for in serum level for AGT to compare the patients with the control group because the TT genotype was absent in patients and controls. And, the CT genotype does not appear in the control. Results suggested that the T allele was a risk allele, and individuals who carry risk alleles have greater plasma angiotensinogen levels and higher risks of developing essential hypertension and, AGT/CC homozygous may be a protective function against development of hypertension.

The results of the present study agreement with the study by Khin Sandar *et al*. (9) were found that *AGT* rs5046 polymorphism was significantly associated with essential hypertension and the mean plasma angiotensinogen level of hypertensive was significantly higher than those of normotensives (p < 0.001). In addition, suggested that the T allele was a risk allele and subjects with risk allele carrier have higher plasma angiotensinogen levels and increased risk of essential hypertension, patients carrying TT genotypes have the higher level of plasma angiotensinogen levels than other genotypes CT and CC

(p=0.005 and p < 0.001) in both hypertensive and normotensives. Al-Hassani (10). A study of Iraqi patients in Mosul City reported an association between *AGT* C/T polymorphism and hypertension. Then, the risk of developing hypertension seems to be higher for the TT genotype and T allele compared to the CC genotype and C allele. Al-Hassani (10) reported the polymorphisms in the promoter region of the angiotensinogen (*AGT*) gene may affect *AGT* transcription and level of blood pressure According to the findings of the current investigation, which were in agreement with a study by Fajar *et al*. (11). *AGT* (rs5046) was associated with a higher risk of developing essential hypertension. And, the *T* allele of *AGT* C/T was found to increase the risk of essential hypertension (p=0.045). While decreasing the risk was found in the C allele p=0.0360.

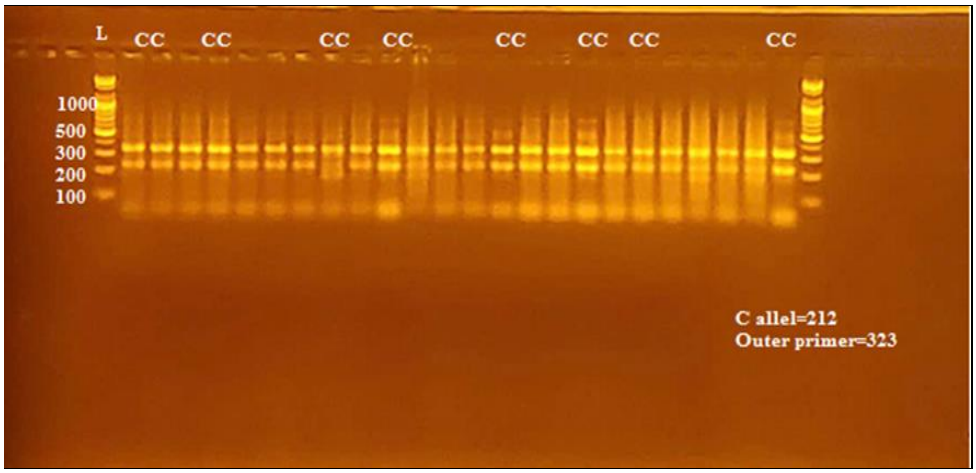

Figure (1): The gel electrophoresis of PCR products of *AGT* gene (rs5046) in apparently healthy control; L: 100 bp DNA Marker; C allele: (212 bp), and two outer primers (323bp) in (2% agarose gel; 50-volt current for 15 min. then 80 volts for 1:30 min.

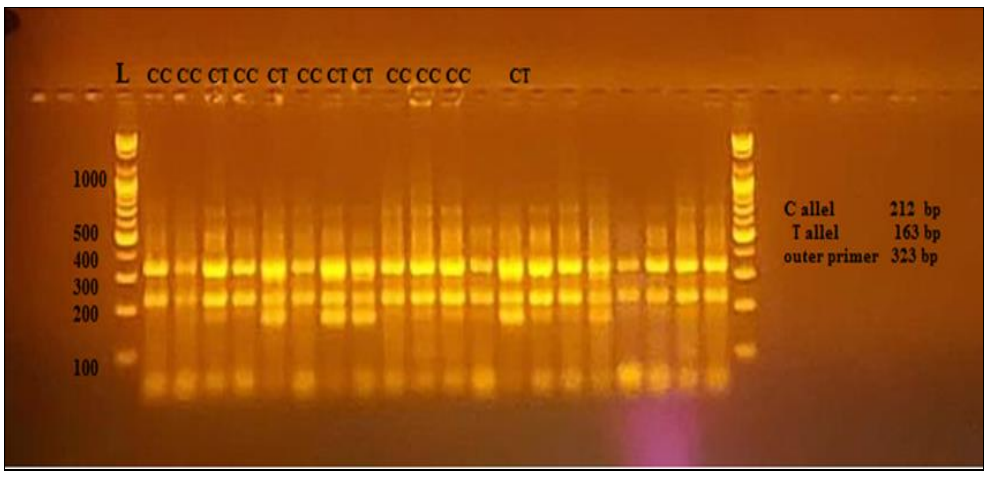

Figure (2): The gel electrophoresis of PCR products of *AGT* gene (rs5046) in patients; L: 100 bp DNA Marker; C allele: (212 bp), T allele (163) and two outer primers (323bp) in (2% agarose gel; 50-volt current for 15 min. then 80 volts for 1:30 min.)

Table (4): The distribution of genotypes and allelic frequency of *AGT* gene polymorphism (5046) in the study group.

Genotypes of rs5046	Patients group No. (%)	Control group No. (%)	OR (95% CI)	EF or PF	Fisher's exact probability
TT	0 (0.0)	0 (0.0)	-	-	-
CT	16 (32.0)	0 (0.0)	48.30 (2.88 – 809.23)	31.7	7.3×10^{-6}
CC	34 (68.0)	50 (100.0)	-	-	-
Total	50 (100.0)	50 (100.0)			
Alleles frequencies					
T	16 (16.0)	0 (0.0)	39.25 (2.35 – 654.72)	15.9	1.6×10^{-5}
C	84 (84.0)	100 (100.0)	0.03	-	1.6×10^{-5}

Table (5): The AGT level median distribution according to the genotyping of rs 5046 of the studied groups

Genotypes of rs5046	ACE Level Mean ± SE (Unit)		Probability
	Patients	Control	
GG	$36.92^{a} \pm 2.62$	$11.88^{a} \pm 0.82$	1.99×10^{-16}
GA	$32.53^{a} \pm 2.41$	$9.71^{a} \pm 0.84$	4.0×10^{-15}
AA	$35.50^{a} \pm 1.92$	$10.71^{a} \pm 0.65$	8.61×10^{-11}

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