AL-QADISIYAH MEDICAL JOURNAL , QAD.MED.J. 20 (1): JANUARY-JUNE 2024
WWW.qmj.qu.edu.iq

REVIEW ARTICLE

Angiotensinogen Gene Polymorphism in Iraqi Patient with Essential Hypertension

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Abstract:

Background

Different genetic and environmental variables contribute to the complicated condition which is known as essential hypertension (EH). The renin-angiotensin-aldosterone system (RAAS) plays a role in controlling blood pressure. In the RAAS, the angiotensinogen (AGT) is a protein that binds to renin to create angiotensin I, the prohormone of angiotensin II (Ang II). The Ang II's precursor and AGT, are responsible for its biological effects. The AGT gene was first implicated as a susceptibility factor in EH in observational cross-sectional descriptive single-center research. Several AGT variants have been recently discovered and investigated in single-center, cross-sectional observational studies. The current research took place between July 2022 and July 2023 at Al-Diwaniyah Teaching Hospital and the Department of Pharmacology and Therapeutics in the College of Medicine at the University of Al-Qadisiyah in Iraq. All the participants were 90 adults who were diagnosed with essential hypertension, spanning an age range from 20 to 70. This study aims to determine the prevalence of polymorphisms in the angiotensinogen gene among Iraqi patients who were diagnosed with essential hypertension.

The Materials and Methods

This study is a cross-sectional study that was carried out on 90 individuals with essential hypertension. Aldosterone, renin, and angiotensinogen levels in the plasma were determined from blood samples given voluntarily by persons undergoing genetic testing. Serum creatinine, urea, uric acid, glucose, and serum lipids were also measured in addition to blood pressure, BMI, and weight.

The Outcomes of the Study

The results indicated in patients with essential hypertension, that the AGT gene A>G (rs699) and C>T(rs5051) had high angiotensinogen levels.

The Conclusion

There was a significant association between these two (rs699, rs5051) and angiotensinogen levels ($P< 0.05$).

Suggestion

Additional research is required to confirm that genetic polymorphisms associated with angiotensinogen levels are significant predictors of hypertension. If this is confirmed, it should be evaluated as a possible hypertension risk in clinical practice.

Keywords: Angiotensinogen (AGT) Gen, polymorphism, essential hypertension, Iraq.

Introduction

Individuals may be diagnosed with hypertension if blood pressure readings are persistently higher than 140 over 90 mm Hg. It is common, yet it can be very harmful if not addressed. The majority of the world's 1.28 billion adults (those aged 30-79) living in low and middle-income countries suffer from hypertension. Nearly half of those who have hypertension are likely unaware of it at 46% according to some estimates [1]. Only 42% of those who need to take medicine for high blood pressure really do so. Only around one to five (21%) of persons with hypertension have it under control. Hypertension is a major contributor to adult mortality around the world. One of the global targets for noncommunicable illnesses is a 33 percent decrease in hypertension prevalence between 2010 and 2030. People with high blood pressure frequently experience no outward symptoms [2]. They cannot be sure unless they get their blood pressure checked. The chance of developing hypertension increases with age, genetics, obesity, inactivity, a diet high in salt, and heavy alcohol intake. Reduced blood pressure has been linked to dietary changes, quitting smoking, and in-

SUBMITTED: 26/JULY/2023 | ACCEPTED: 10/SEPTEMBER/2023 | PUBLISHED: 30/JUNE/2024

creased physical activity. Some people may still need to take medicine. In the medical field, blood pressure is represented by two numbers [3]. The first number represents the systolic pressure in the arteries, which occurs when the heart contracts. Diastolic blood pressure is the pressure in the arteries when the heart is at rest (during diastole). Hypertension (HTN) is diagnosed when the systolic blood pressure reading is greater than 140 mm Hg and/or the diastolic blood pressure reading is greater than 90 mm Hg on at least two distinct occasions. Cardiovascular disease, kidney disease, nervous system disease, and early mortality are all exacerbated by HTN [4].

Blood pressure (BP) in the arteries can be affected by genetic variations in the AGT gene [5]. In the RAAS, AGT is a protein that binds to renin to generate AGT I, the prohormone of AGT II. By increasing vascular tone and encouraging salt retention, angiotensin II plays a crucial role in BP management [6]. Hepatocytes are responsible for the synthesis and constitutive secretion of the AGT protein into the plasma, extracellular and cerebrospinal fluids, kidney, and adipose tissues [6]. The adventitia and the smooth muscle cells of the vascular walls both contain the AGT protein. Synthesis and secretion of AGT protein are stimulated by glucocorticoids (particularly dexamethasone), estrogens, AGT II, and thyroid hormones [6].

The significance of AGT gene polymorphism in human essential HTN was initially recognised by Jeunemaitre et al [5]. In linkage analyses, researchers discovered a 17% increase in the frequency of the AGT gene in hypertensive siblings. Based on the results of this investigation, mutations in the AGT gene can be a risk factor for HTN in 3–6% of people under the age of 60. In a sample of 63 British families, Caulfield et al. [7] found a 25% higher than expected concordance between the AGT gene and essential HTN. Since then, additional AGT gene variations associated to HTN were found (for example rs4762/T174M, rs699/T235M, rs5050/A-20C, rs5049/G-217A, rs5051/A6G, rs5046/C532T, rs2493134/G507A, rs2493132/C6309A, rs7079/C11535A, rs943580/A1240G, and others). The rs5050/A-20C and rs5049/G-217A polymorphisms may influence the risk of HTN, while data on the rs4762/T174M variant are less consistent, and the rs5051/A-6G polymorphism's effect on hypertension may be more pronounced in populations with a salt-rich diet [8]. These findings resulted from four meta-analyses that included a total of 26,818 people.

The AGT polymorphisms rs5051/A-6G, rs5050/A-20C, C3389/T (rs4762/T174M), and C4072/T (rs699/T235M) were analyzed by Li et al [9]. They found an increased incidence of essential HTN in a Northern Han Chinese population with the AGT -6A/A and 4072C/C polymorphisms. The current research was published in the current issue of Angiology. To determine whether the polymorphisms rs5051/A-6G, rs5050/A-20C, and rs5049/G-217A were associated with essential HTN in the Chinese population, Xi et al. conducted a meta-analysis (7966 patients) [10]. Although rs5051/A-6G polymorphism has been linked to an elevated incidence of essential HTN in Western populations, Li's recent meta-analysis (9306 patients) indicates that this may not be the case in a Chinese population [11].

The Materials and Methods

Study Design, Patients Recruitment, Setting and Timing

The patients in this study had been diagnosed with HTN according to ESH 2023, and the study design is observational and cross-sectional descriptive. Each potential participant was evaluated and selected by a cardiologist or other cardiology professional. The research took place between July 2022 and July 2023 at the Department of Pharmacology and Therapeutics at the College of Medicine at Al-Qadisiyah University. Before enrolling in the study, all 90 patients would be provided with written and verbal information on the procedure and the goals of the research. The participants ranged in age from 20 to 70, and all had been diagnosed with essential HTN.

The Questionnaire

Information was collected from the patients including name, age, sex, race, weight, length, BMI, SBP, DBP, and comorbidities (smoker, DM, IHD). Biochemical parameters were measured for all the patients with valsartan which were urea, creatinine, uric acid, glucose, serum lipid (LDL, HDL, Total cholesterol, Triglyceride, Atherogenic index), Aldosterone level, Renin level, Aldosterone / Renin ratio, and Angiotensinogen level.

The Exclusion Criteria

The following criteria were used to exclude some individuals.

- Impairment of the kidneys or liver.
- Pregnancy.
- Heart failure.
- Obesity (BMI ≥ 30).
- Psychiatric patient.

The Subjects

The study included 90 adults enrolled in this study (37 males and 53 females) aged (20-70) years and were diagnosed with essential HTN.

The Ethical Considerations

The study was authorized by the Ethics Committee of the College of Medicine at Al-Qadisiyah University; all the patients were given information about the study methods, and they previously gave their consents.

The Blood Samples

4 ml of blood had been drawn from the patients' antecubital veins; 1 ml of the blood was placed in an EDTA-treated tube for DNA extraction and held at -20º C until the time of DNA extraction. The remaining 3 ml of blood was poured into a gel tube, spun for 5 minutes at 5,000 RPM to separate the serum, and then used in biochemical analyses.

Checking the Blood Pressure

The BP of patients was measured with a mercury sphygmomanometer. The respondents were instructed to sit quietly for 5 minutes with their legs uncrossed and their right arms exposed in order to take accurate measurements. The right arm was then laid out flat on the table, palm facing up. The correct sleeve size was chosen. All the measurements were taken with the cuff at the same level as the heart.

The patients were classified according to the ESH as in Table 1.

Blood Pressure Category	Systolic Blood Pressure(mmHg)	Diastolic Blood Pressure(mmHg)
Optimal	<120	and <80
Normal	120-129	and 80-84
High normal	130-139	and /or 85-89
Stage 1 hypertension	140 – 159	and /or 90-99

Stage 2 hypertension	160-179	and /or 100-109
Stage 3 hypertension	≥180	and /or ≥110
Isolated systolic hypertension	≥140	And<90
Isolated diastolic hypertension	<140	and ≥90

The score for BP control or response [12]

a) Good responders were defined as patients that achieved a target BP in (SBP < 140 mm Hg or DBP < 90 mm Hg).
b) Moderate responders were defined as patients whose BP was 150/100 mm Hg.
c) Poor responders were defined as patients whose BP was > 150/100 mm Hg.

The PCR – TETRA ARM Technique

Human blood samples were analyzed using the PCR-TETRA ARM method for genotyping and identifying the AGT (rs5051) and AGT (rs699) gene polymorphisms. The polymerase chain reactions (PCR) were carried out in sterile 25 l PCR tubes. DDH2O was used to bring the total volume of the reaction mixture to 25 l, and the concentrations of the reactants were optimized (MgCl2 1.5 mM, Taq polymerase 1 U, each dNTP 200 M). A negative control blank was included in each round of amplification to serve as a reference point for results. To ensure that the final volume of the reaction was 25 l, the mixture was centrifuged for 3 seconds to collect the drops from the wall, and then the extracted DNA was subjected to amplification. The product of the amplification reaction was detected by running the samples through electrophoresis of 2% agarose at 50 volts for 2 hours while using a (1000 bp) DNA ladder as a size marker.

The PCR Pre-Mix Preparation

PCR per-mix for the gene was prepared by using (Accu Power PCR Pre Mix Kit) according to the instructions of the company as in Table 2:

Table 2: PCR Pre Mix

PCR Pre Mix	Volume
DNA template	5 µl
Forward inner (10pmol/ µl)	1 µl
Forward outer (10pmol/ µl)	1 µl
Reverse inner (10pmol/ µl)	1 µl
Reverse outer (10pmol/ µl)	1 µl
PCR water	11 µl
Total volume	20 µl

After that, these PCR Pre-Mix components were placed in a standard Accu Power PCR Pre-Mix Kit which contains all other components that are required for PCR reaction such as Top DNA polymerase, dNTPs, Tris-HCl(pH: 9.0), KCl, $MgCl_2$, stabilizer, and tracking dye.

Then, all the PCR tubes were transferred into a vortex centrifuge to dissolve the lyophilized blue pellet and then briefly spin down. Then they were placed in a PCR Thermocycler (SimpliAmp. USA).

The PCR Thermocycler Condition

The PCR thermocycler conditions were done for the angiotensinogen (AGT) gene as it is shown in Table 3

Table 3: The PCR thermocycler conditions

PCR step	Temp.	Time	Repeat
Initial denaturation	95°C	5min.	1
Denaturation	95°C	1min.	35cycle
Annealing	63(*) °C	1min.	
Extension	72°C	1min.	
Final extension	72°C	7min	1

(*) annealing temp. for both AGT (rs5051) and AGT (rs699)

The PCR Product Analysis

Agarose gel electrophoresis was used to examine the PCR products, yielding the results that are depicted in Figures (1 and 2) and the following:

1. 1% Agarose gel was made by mixing 100 ml of 1X TBE (work solution) with 1 gram of agarose powder.

The agarose gel solution was then stained with 3 L of ethidium bromide and heated in a microwave for 10 minutes. The mixture was heated until the sugar dissolved and then cooled to room temperature.

To make 1X TBE, 10X TBE is diluted to a 1:9 ratio with distilled water.

2. An agarose gel solution was placed in a tray and left to solidify at room temperature for 15 minutes before the comb was carefully removed. Finally, 10ul of PCR product and 10ul of (100 bp Ladder) were added to the first well of the comb.

3. 1X TBE buffer was added to the electrophoresis chamber and the gel tray was placed there. Then, for an hour, a current of 100 volts and 80 milliamperes was applied.

4. A UV transilluminator was used to observe the PCR products.

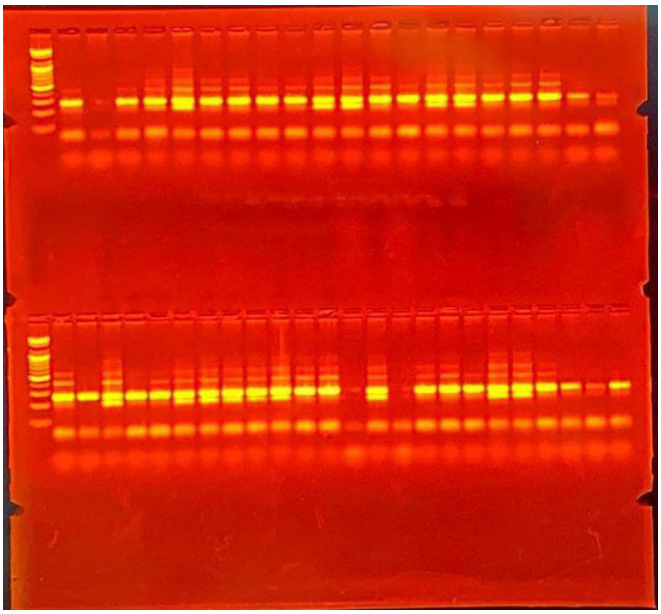

Figure 1: The AGT(rs5051) gene was analyzed in PCR and the results were visualized on an agarose gel electrophoresis.

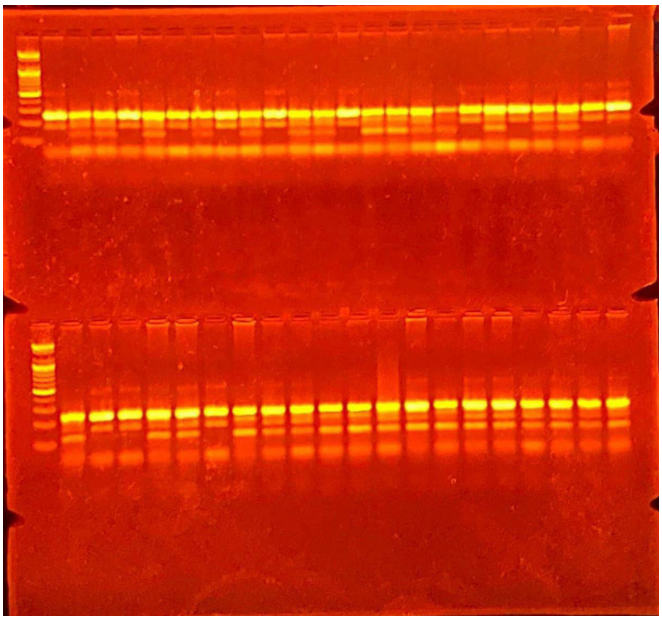

Figure 2: AGT(rs699) gene PCR product analysis in agarose gel electrophoresis from a subset of human blood samples.

The Results

The Demographic Data

This study included 90 Iraqi hypertensive patients, (58.9%) (n = 53) females and (41.1%) (n = 37) males, with a mean age of ± SD age (years) 53.2 ± 13.8 years old, and mean BMI ± SD BMI 29 ± 5. 3.2 kg/m². As in Figure (3).

The Metabolic Profile

Among the study patients, the mean ± SD of total serum cholesterol, triglyceride, atherogenic index, and uric acid was 195.8 ± 44.8 mg/dL, 223.7 ± 105.7 mg/dL, 0.7 ± 0.3, 5.2 ± 1.5 mg/dL respectively. as it is shown in tables (4) and (5).

Figure (3): The pie chart illustrates the sex distribution of participant hypertensive patients in the study (n = 90).

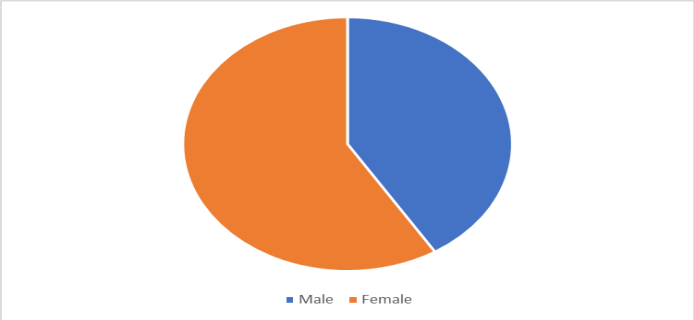

Table (4): Illustrating values of blood urea and serum creatinine of recruited hypertensive patients (n = 90).

	Minimum	Maximum	Mean	Standard Deviation SD
Blood urea	16.8	60	36.9	9.3
Creatinine	0.45	1.4	0.9	0.2

Table (5): Illustrating the metabolic profile characteristics of recruited hypertensive patients enrolled in the study.

	Minimum	Maximum	Mean	Standard Deviation (SD)
Cholesterol	105.0	357.0	195.9	44.8
Triglyceride	91.0	722.0	223.7	105.7
Atherogenic Index	0.3	2.7	0.7	0.3
Uric acid	2.9	12.5	5.2	1.5

The Blood Pressure

All patients who participated in this study were hypertensive, so the mean ± SD of systolic blood pressure was 150±14.9 mmHg while the mean ± SD of diastolic blood pressure was 89.3± 6.9 mmHg. Of these 90 hypertensive patients (45.6 %) (n = 41) met the target level of BP (responders<140/90 mmHg) while (54.4 %) (n = 49) did not meet the target of treatment (≥ 140 mmHg non-responders) (Figure 4).

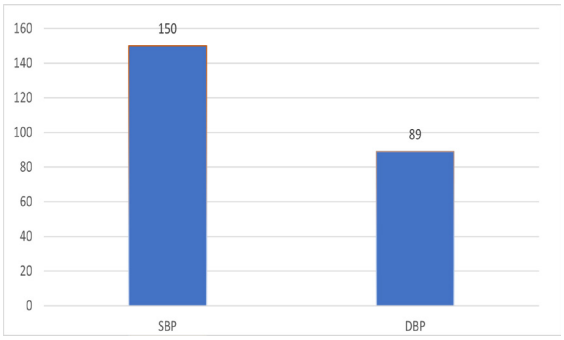

Figure (4): Shows the mean systolic and diastolic blood pressure of recruited hypertensive patients (n = 90). Systolic blood pressure (SBP), Diastolic blood pressure (DBP).

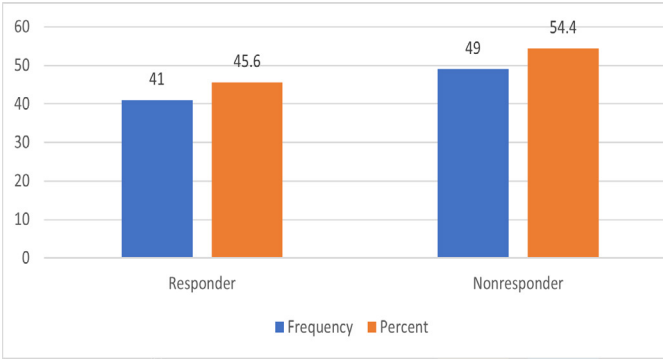

Figure (5): Genotyping frequency of AGT gene rs699 among Iraqi hypertensive patients.

Alleles and Genotype Frequency of AGT gene rs699 and rs5051

Table 6 and Figures 5 and 6 display the allele and genotype frequencies of AGT across all the patients who participated in this investigation. The most common rs699 genotype was AG (44%), whereas the most common rs699 allele was G (67%). The allele C for rs5051 was found to be the most common (98 out of 54%), with the corresponding genotype being CT (46 out of 51%). There was no statistically significant deviation from the Hardy-Weinberg equilibrium frequency distribution (P>0.05).

Table (6) Genotype Frequency of AGT Gen polymorphism among Iraqi hypertensive patients.

	Genotype	Actual		Expected by Hardy-Weinberg law		P value
		Number	Frequency	Number	Frequency	
699	AA	8	0.09	10	0.11	0.8(NS
	AG	44	0.49	40	0.44	
	GG	38	0.38	40	0.44	
	Total	90	1.000	90	1.00	
	Allele					
	A	60	0.33	NA	NA	
	G	120	0.67	NA	NA	
	Total	180	1.00			
5051	TT	18	0.2	19	0.21	0.14 (NS)
	CT	46	0.51	45	0.49	
	CC	26	0.29	26	0.3	
	Total	90	1.00	90	1.00	
	Allele					
	T	82	0.46	NA	NA	
	C	98	0.54	NA	NA	
	Total	180	1.00			

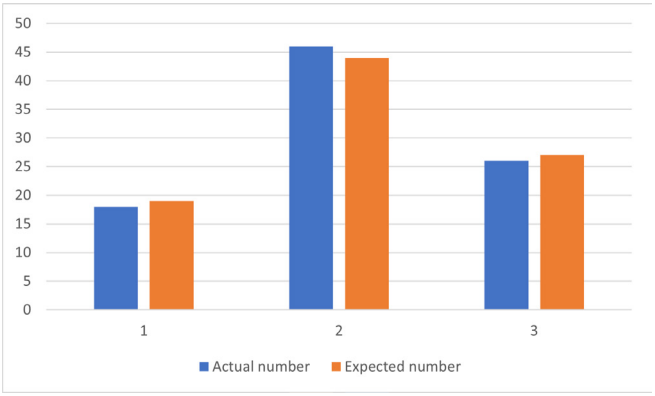

Figure (6): Genotyping frequency of AGT gene rs5051 among Iraqi hypertensive patients.

The Effect of AGT Polymorphism rs699 (M235T) on Angiotensinogen Level

Angiotensinogen plasma levels were found to be greater in homozygous GG carriers (4.2 0.15 ng/dl) than in heterozygous AG carriers (2.6 0.13 ng/dl), as it is reported in Table (7). Angiotensinogen levels in the plasma of GG carriers were significantly greater than those of AA and AG carriers (P 0.001). In addition, the plasma Angiotensinogen level was significantly higher in AA carriers than in AG carriers (P 0.001). The plasma aldosterone levels were significantly different between the two groups.

The Effect of AGT Polymorphism rs5051 (G-6A) on Angiotensinogen Level

Table 8 shows that the average plasma concentration of Angiotensinogen was higher in CC carriers (4.4 0.16 ng/dl) than in TT carriers (2.9 0.3 ng/dl). Angiotensinogen levels in the plasma of TT carriers were significantly lower than those of CC carriers (P 0.001), while Angiotensinogen levels in the plasma of CT carriers were significantly greater than those of TT carriers (P

0.001). However, the plasma levels of Angiotensinogen differed significantly (P 0.001) between those with the homozygous TT genotype and those with the heterozygous CC genotype.

Table (7): Values of mean ± SE of Angiotensinogen level and genotyping frequency in Iraqi hypertensive patients of AGT Gen Polymorphism rs699 (M235T).

Genotype rs699 (M235T).	Numbers	Mean Angiotensinogen level (ng/dl)	S. E	P value
AA	8	2.9	0.5	<0.001
AG	44	2.6	0.13	
GG	38	4.2	0.15	

Table (8): Values of mean ± SE of Angiotensinogen level and genotyping frequency in Iraqi hypertensive patients of AGT Gen polymorphism rs5051 (G-6A).

Genotype rs5051 (G-6A)	Numbers	Mean Angiotensinogen level (ng/dl)	S.E	P value
CC	26	4.4	0.16	<0.001
CT	46	3.1	0.12	
TT	18	2.9	0.3	

The Discussion

The Allele Frequency and Genotyping of rs699 and rs5051

The current study is a cross-sectional analysis of AGT rs699 and rs5051 polymorphisms in Iraqi patients with EH.

The most common rs699 genotype was AG (44%), while the most common rs699 allele was G (67%). while AA genotype was at 9% (p= 0.8) and A allele frequency was at 33%.

The C allele was the most common at rs5051 (54%), and the CT genotype was the most common at 51%. while the frequency of the T allele was low (46%), at just 29% (p=0.14(·

The AGT gene contains a single-nucleotide polymorphism (SNP) known as Rs699. Despite being referred to as “M235T” or “Met235Thr” in the vast majority of published works, this SNP actually refers to a difference in amino acid 268 (not 235) in modern databases. C4072T [13] is another name for rs699. Greater plasma AGT levels and, thus, greater BP and an increased risk for hypertension-related diseases are associated with the rs699(C) allele, which encodes the threonine variation. The rs5051(T) allele, as aligned to the db SNP entry, not as published, is associated with an elevated risk for hypertension and complications [14], likely as a result of its tight connection with rs699. Higher plasma angiotensinogen levels are linked to rs699(T), which in turn increases the risk of EH. Similar to the increased prevalence of HT in African communities, Africans seem to have higher rates of the rs699(T) gene than Caucasians do. Rs 5051 also goes by the moniker “A-6G” [15].

The Genotype /Phenotype Relationship and the Impact of AGT Gene rs699 and rs5051 on Blood Pressure

Patients with homozygous AA, heterozygous AG, and homozygous GG alleles had systolic BP readings of 154 3.4, 148 2.2, and 151 2.9, respectively (P = 0.7). When comparing the diastolic BP of the patients who were homozygous AA, heterozygous AG, and homozygous GG carriers, the P value was 0.08. AGT rs699 did not influence BP on either the systolic or diastolic side.

The patients who were either homozygous for CC alleles or heterozygous for CT alleles or homozygous for TT alleles had systolic BPs of 144/4.7, 152/1.9, and 149/2.9, respectively (P = 0.3). However, the average diastolic BP of CC homozygotes, TT heterozygotes, and TT homozygotes was 88/1.4/90/1.2. The significance level was 0.6. AGT rs5051 did not have a detectable impact.

No association was discovered between either systolic or diastolic BP and either polymorphism (rs5051 C>T or rs699 A>G) in this investigation of Iraqi patients with essential hypertension.

An impact magnitude four times larger than that found in White participants [16,17] was seen between rs5051 C>T and/or rs699 A>G and systolic blood pressure in Black people in the UK Biobank. Allele frequencies for rs699 A>G and rs5051 C>T were around twice as common in persons of African ancestry as they were in people of European ancestry, respectively. In addition to the differences in minor allele frequency, it is known that Whites and Blacks differ in the linkage disequilibrium blocks of AGT [18]. Consistent with the observation that salt-sensitive hypertensive phenotypes were more common in people of African ancestry, there is evidence to indicate that rs5051 C>T and rs699 A>G have a more profound impact on blood pressure changes in Black patients due to differences in AGT expression or abundance [19]. Plasma renin activity is reduced and aldosterone levels are maintained with the use of calcium channel blockers and diuretics, whereas it is increased with the use of beta-blockers or RAS acting antihypertensives in people of African ancestry [20, 21]. The JNC 8 guidelines, which

call for separate first antihypertensive treatment plans for Caucasian and Black patients, are followed here. In Caucasians with normal renal function, the JNC only recommends angiotensin-converting enzyme inhibitors as first-line therapy [22,23]. Here, we report data suggesting that the rs5051 C>T and rs699 A>G genetic variations can play a role in explaining the known differences in the response to antihypertensive medications between people of European and African ancestry. Despite this, extensive interbreeding of populations occurs. When whole genome sequencing is more commonly available in clinical treatment, doctors will be able to stop utilizing racial identification as a stand-in for genotype [24].

In this study, there is no evidence linking the AGTR1 1166 A/C polymorphism or the ACE I/D polymorphism to BP responses. An improved BP response had been linked to the AGT 235Thr allele when treated with angiotensin-converting enzyme (ACE) inhibitors or beta-blockers [25,26], while other studies have found no gene-drug interaction when treating hypertension with ACE inhibitors, beta-blockers, calcium channel antagonists, or angiotensin receptor blockers. Consistent with these other studies, the present study did not find evidence connecting the AGT Met235Thr genotype and BP response to a specific medication for HPT[27].

The Conclusion

The current study results showed that the most common allele for rs699 was the G allele (67%) while the most frequent genotype was AG (49%), frequency of another genotypes GG and AA were 38% and 8% respectively. Regarding rs5051 the most frequent allele was C (54%) while the most frequent genotype was CT (46%), frequency of other genotypes TT and CC were 2% and 29% respectively. This study demonstrated a significant association between two of these polymorphisms and hypertension.

Reference

1. World Health Organization. 2018. Key Facts about Hypertension in Iraq.

2. Saka M, Shabu S and Shabila N. Prevalence of hypertension and associated risk factors in older adults in Kurdistan, Iraq. East Mediterr Health J, 2020; 26: 268-75.

3. World Health Organization, "Urbanization and health," Bulletin of the World Health Organization, 2022; 88(4): 245-246.

4. Li, H., et al. The relationship between angiotensinogen gene polymorphisms and essential hypertension in a Northern Han Chinese population. Medical Journal of Babylon, 2014; 65(7): 614-619.

5. Xi, B., et al. Association of polymorphisms in the AGT gene with essential hypertension in the Chinese population. Hypertension, 2012; 13(2): 282-288.

6. Li Y.-y.J.J.o.C.M. Lack of association of A-6G polymorphism of AGT gene with essential hypertension in the Chinese population. Oncogene, 2012; 13(8): 505-510.

7. Baker, M., et al. The C-532T polymorphism of the angiotensinogen gene is associated with pulse pressure: a possible explanation for heterogeneity in genetic association studies of AGT and hypertension. Nature, 2007; 36(6): 1356-1362.

8. Gu C.C., et al. Haplotype association analysis of AGT variants with hypertension-related traits: the HyperGEN study. J Clin Invest, 2005; 60(3): 164-176.

9. Balam-Ortiz, E., et al. Hypercontrols in genotype-phenotype analysis reveal ancestral haplotypes associated with essential hypertension. EMBO J, 2012; 59(4): 847-853.

10. Wang, W.Y., et al. Exclusion of angiotensinogen gene in molecular basis of human hypertension: Sibpair linkage and association analyses in Australian Anglo-Caucasians. Hypertension, 2016; 87(1): 53-60.

11. GBD Mortality and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death. Lancet, 2016; 388: 1459-544.

12. Gardemann, A., Stricker, J., Humme, J. et al. Angiotensinogen T174 M and M235T gene polymorphism are associated with the extent of coronary atherosclerosis. Am J Hypertens, 2011; 145(2): 309-314.

13. Unniachan, Sreevalsa, David, et al. Evaluation of blood pressure reduction response and responder characteristics to fixed-dose combination treatment of amlodipine and losartan: a post hoc analysis of pooled clinical trials. Journal of Personality and Social Psychology, 2014; 16(9): 671-677.

14. Yugar-Toledo JC, Martin JF and Krieger JE. Gene variation in resistant hypertension: multilocus analysis of the angiotensin 1-converting enzyme, angiotensinogen, and endothelial nitric oxide synthase genes. Indian Journal of Preventive and Social

Medicine, 2011; 30(8): 555-564.

15. Yako YY, Balti EV and Matsha TE. Genetic factors contributing to hypertension in African-based populations: A systematic review and meta-analysis. Nature, 2018; 20(3): 485-495.

16. Purkait P, Halder K and Thakur S. Association of angiotensinogen gene SNPs and haplotypes with risk of hypertension in eastern Indian population. Oncogene, 2017; 23: 1-10.

17. Liu C, Kraja AT and Smith JA. Meta-analysis identifies common and rare variants influencing blood pressure and overlapping with metabolic trait loci. The journal of biological chemistry, 2016; 48(10): 1162-1170.

18. Bis JC, Smith NL and Psaty BM. Angiotensinogen Met235Thr polymorphism, angiotensin-converting enzyme inhibitor therapy, and the risk of nonfatal stroke or myocardial infarction in hypertensive patients. Behavior Therapy, 2003; 16(12): 1011-1017.

19. Do AN, Lynch AI and Claas SA. The effects of genes implicated in cardiovascular disease on blood pressure response to treatment among treatment-naive hypertensive African Americans in the GenHAT study. Oncogene, 2016; 30(9): 549-554.

20. Liljedahl U, Kahan T and Malmqvist K. Single nucleotide polymorphisms predict the change in left ventricular mass in response to antihypertensive treatment. Hypertention, 2004; 22(12): 2321-2328.

21. Kurland L, Melhus H and Karlsson J. Polymorphisms in the angiotensinogen and angiotensin II type 1 receptor gene are related to change in left ventricular mass during antihypertensive treatment: results from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation versus Atenolol (SILVHIA) trial. Am J Hypertens, 2002; 20(4): 657-663.

22. Kurland L, Liljedahl U and Karlsson J. Angiotensinogen gene polymorphisms: relationship to blood pressure response to antihypertensive treatment: results from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation vs Atenolol (SILVHIA) trial. Behavior Therapy, 2004; 17(1): 8-13.

23. Inoue I, Nakajima T and Williams CS. A nucleotide substitution in the promoter of human angiotensinogen is associated with essential hypertension and affects basal transcription in vitro. Oncotarget, 2017; 99(7): 1786-1797.

24. Mopidevi B, Kaw MK and Sivankutty I. A polymorphism in intron I of the human angiotensinogen gene (hAGT) affects binding by HNF3 and hAGT expression and increases blood pressure. Hypertention, 2019; 294(31): 11829-11839.

25. Bonnardeaux A, Davies E, Jeunemaitre X, et al. Angiotensin II type 1 receptor gene polymorphisms in human essential hypertension. Hypertention, 2014; 24(1): 63-69.

26. Jeunemaitre X, Soubrier F and Kotelevtsev YV. A mutation of angiotensinogen in a patient with preeclampsia leads to altered kinetics of the renin-angiotensin system. J Hypertens, 2015; 270(19): 11430-11436.

27. O'Donnell CJ, Lindpaintner K and Larson MG. Evidence for association and genetic linkage of the angiotensin-converting enzyme locus with hypertension and blood pressure in men but not women in the Framingham Heart Study. Hypertention, 2018; 97(18): 1766-1772.