ORIGINAL ARTICLE

CONTENTS

Genetic polymorphism of the CYP11B2 gene in an Iraqi patient with essential hypertension

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ABSTRACT

Aim: To demonstrate the genetic variant of CYP11B2 Gen rs1799998 and rs4539 and their effect on systolic and diastolic blood pressure in Iraqi patient with essential hypertension in Aldiwania province.

Materials and Methods: This is an observational cross sectional descriptive single centre study for hypertensive patients at Al-Diwaniyah province, Iraq which is diagnosed according to 2020 ISH. All candidate patients were diagnosed and recruited by specialist caregiving physician/ cardiologist. There was a total of 90 participants, 37 males and 53 women. Aldosterone and renine levels in the plasma were determined from blood samples given voluntarily by patients undergoing genetic testing.

Results: Regarding rs4539 the most frequent allele was T (112, 62%) while the most frequent genotype was TC (54, 60%). Regarding rs1799998 the most frequent allele was G (97, 54%) while the most frequent genotype was AG (49, 54%).

Conclusions: There was no significant relationship between rs1799998 (A344>G) and rs4539 (T2718C) with systolic and diastolic blood pressure in Iraqi patient with essential hypertension.

KEY WORDS: CYP11B2 gen, polymorphism, essential hypertension, Iraq

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INTRODUCTION

Hypertension is office/clinic blood pressure consistently over 140/90 mm Hg (systolic and/or diastolic) that needs long-term management [1]. In general, human blood pressure fluctuates naturally throughout the day and is problematic if it remains constant [2]. Hypertension can be categorized as follows: 1) essential hypertension (primary) occurs in approximately 95% of patients, in which the direct causes are unknown; 2) secondary hypertension 5% to 10% of the hypertensive population have identifiable causes, such as hormonal problems, large blood vessel narrowing problem, or kidney vessel narrowing [3]. In low and middle-income nations, hypertension is a leading cause of cardiovascular illness and death worldwide [4]. Obesity, insulin resistance, excessive alcohol consumption, excessive sodium consumption (in sodium-sensitive patients), advanced age, inactivity, stress, inadequate potassium and calcium intake, and irregular heartbeat are all contributors to hypertension [5]. In the endocrine mechanism of the RAAS (renin-angiotensin-aldosterone system), renin is responsible for the conversion of angiotensinogen to angiotensin I [6], and angiotensin I is converted to angiotensin II (Ang II) under the action of angiotensin-converting enzyme. The most potent blood artery constriction agent is angiotensin II (Ang II), which is produced from angiotensin I by angiotensin converting enzyme. Arterial smooth muscle is affected, peripheral resistance is increased, and blood pressure is increased. In addition to increasing blood volume and blood pressure, angiotensin II also induces the adrenal glands to secrete aldosterone, which stimulates the epithelial cells of the kidneys to promote sodium and water reabsorption. The possibility that mutations in genes encoding enzymes involved in aldosterone synthesis led to elevated aldosterone levels and, eventually, essential hypertension [7]. The CYP11B2 gene encodes aldosterone synthase on chromosome 8q22. The gene contains 9 exons and spans approximately 7000 base pairs of DNA [8]. Aldosterone synthase is an enzyme belonging to the cytochrome P450 superfamily. The enzyme CYP11B2 lies within the mitochondrial inner membrane. The CYP11B2 enzyme, also known as three consecutive processes are catalyzed by aldosterone synthase. In the synthesis of aldosterone from 11-deoxycorticosterone [9], there are a number of CYP11B2 polymorphisms that have been linked to elevated CYP11B2 transcription, elevated aldosterone

production, and the development of a wide variety of cardiovascular problems [10]. The 344 C/T polymorphism in the 5' promoter region of the CYP11B2 gene is the first and most extensively researched polymorphism. Increased aldosterone synthesis and secretion in serum or urine, as well as an elevated aldosterone renin ratio, have both been linked to the CYP11B2 344C/T polymorphism. The glycine-to-arginine (K173R) missense mutation in exon 3 (rs 4539) is another intriguing polymorphism linked to elevated aldosterone production [10].

AIM

The aim of this research is to demonstrate the genetic variant of CYP11B2 Gen rs1799998 and rs4539 and their effect on systolic and diastolic blood pressure in Iraqi patient with essential hypertension in Al-Diwaniyah province.

MATERIALS AND METHODS

STUDY DESIGN

This is an observational cross sectional descriptive single centre study for hypertensive patients of Iraqi nationality, which is diagnosed according to ISH 2020. All candidate patients were diagnosed and recruited by the specialist caregiving physician/cardiologist. The research was carried out at the Al-Diwaniyah Teaching Hospital and the Department of Pharmacology and Therapeutics at Al-Qadisiyah University, College of Medicine, Iraq. It extended from July 2022 through July 2023. The lab work was completed in Department of Pharmacology and Therapeutics, College of Medicine, Al- Qadisiyah University, at Al-Diwaniyah province.

SUBJECTS

The study included 90 adults (36 male and 54 female) aged 20-70 years diagnosed with essential hypertension, taking valsartan for at less two weeks.

ETHICAL CONSIDERATIONS

The study was approved by the Ethics Committee of the College of Medicine, University of Al- Qadisiyah and procedures were explained to all patients and informed consent was taken from all patients.

PRIMERS USED IN THE CURRENT STUDY

Polymerase chain reaction (PCR) primers for *CYP11B2 rs1799998* and *CYP11B2 rs4539* gene had been given by Bioneer Company, Korea (Table 1).

CHEMICALS USED IN THE CURRENT STUDY

In this analysis, we used a variety of chemicals have been demonstrated with the corresponding country of origin and manufacturing company (Table 2).

COLLECTION AND PREPARATION OF SAMPLES

Blood sample: 1 ml blood samples were collected from the patients that were aspirated from antecubital vein. 1 ml blood was placed until the time of DNA extraction, kept in 1 ml an EDTA tube at -20 C.

GENOTYPING

Genomic DNA was extracted by using Genaid DNA extraction kit (USA).

PCR-TETRA ARM TECHNIQUE

The PCR-TETRA ARM technique was performed for genotyping and detecting *CYP11B2 (rs1799998)* and *CYP11B2 (rs4539)* gene polymorphism in blood samples of human. PCR reactions were performed by using Accupower kit (Bioneer, Korea).

PCR PreMix preparation: PCR per-mix for the gene was prepared by using AccuPower PCR PreMix Kit according to the instructions of the company.

PCR Thermocycler condition: The PCR thermocycler conditions were done for the *CYP11B2* gene according to SimpliAmp (USA), as shown in table 3**.**

PCR product analysis: Agarose gel electrophoresis was used to examine the PCR results as per protocol (MarLiJu, Korea**)** as shown in fig. 1, fig. 2.

STATISTICAL PROCESSING

A mean and standard deviation (SD) were used to represent the data. Statistical analysis was performed using SPSS version 26. Analysis of variances (one-way ANOVA) was used to compare more than two means. The Fissure exact test and the Chi-square were used to see if there was a significant difference in demographic data between the two sets of categorical data. The allele frequencies of hypertensive patients and the aldosteron level, were compared using odds ratios (ORs) and 95% confidence intervals (CIs). It was found that $p \leq 0.05$ was the statistically significant in all statistical analyses in this study. The Hardy–Weinberg law state that the allele and genotype ferequencies in apopulation will remain constant from generation to generation in the absence of evolutionary influences.

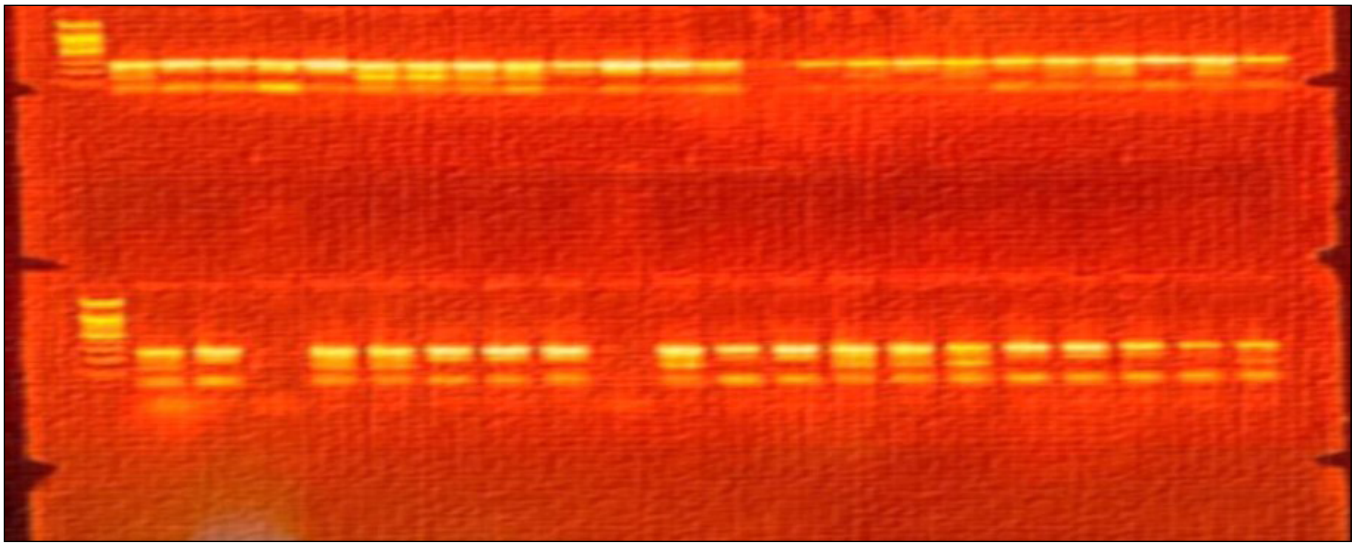

Fig. 1. The PCR product of CYP11B2 (rs1799998) gene.

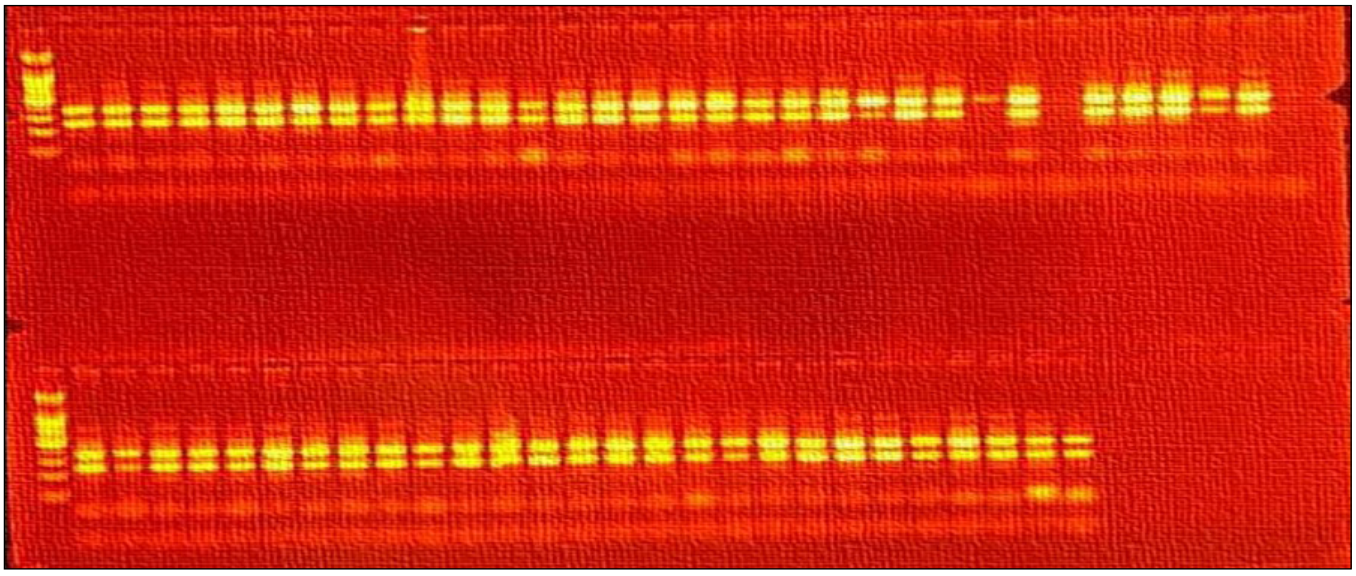

Fig. 2. The pcr product of CYP11B2 (rs 4539) gene.

RESULTS

FREQUENCY OF ALLELES AND GENOTYPES OF THE CYP11B2 GENE RS4539 AND RS1799998

The genotypes of all participants in this study and the allele frequencies of CYP11B2 are shown in table 4.

Regarding rs4539 the most frequent allele was T (112, 62%) while the most frequent genotype was TC (54, 60 %). Regarding rs1799998 the most frequent allele was G (97, 54%) while the most frequent genotype was AG (49, 54 %). There was no significant difference between the actual and expected frequency distribution ($P > 0.05$).

The effect of CYP11B2 rs1799998 (A344>G) and rs4539 (T2718C) polymorphism on systolic and diastolic blood pressure in Iraqi hypertensive patients taken valsartan 160 mg/day ($P>0.05$) as shown in table 5 and table 6.

This study was investigated the polymorphism of CYP11B2 gen rs1799998 and rs4539 in Iraqi patient with essential hypertension in Al-Diwaniyah province. Regarding rs1799998 the most frequent allele was G (54%), GG (27%) while the most frequent genotype was AG (54 %). The A allele have minor frequency 46% and AA genotype 19%. There was no significant difference between the actual and expected frequency distribution ($P > 0.05$). The mean ± SE systolic blood pressure in homozygous AA, heterozygous AG and homozygous GG carrier patients was 152 ±3.2, 150±2.2, 144±2.8 respectively. On other hand mean diastolic blood pressure in homozygous AA, heterozygous AG and homozygous GG carrier patients was 91± 1.7, 89 ± 1, 86 ± 1.1 respectively. There was no statistically significant effect of CYP11B2 rs1799998 on systolic and diastolic blood pressure.

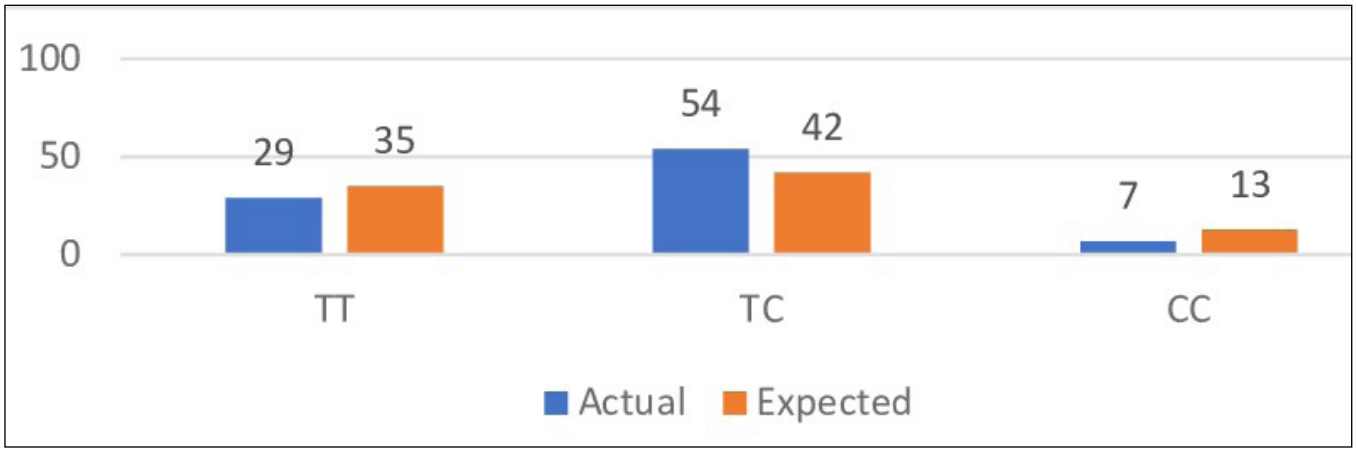

Fig 3. Genotyping frequency of CYP11B2 Gene rs4539 among Iraqi hypertensive patients taken valsartan 160 mg/day.

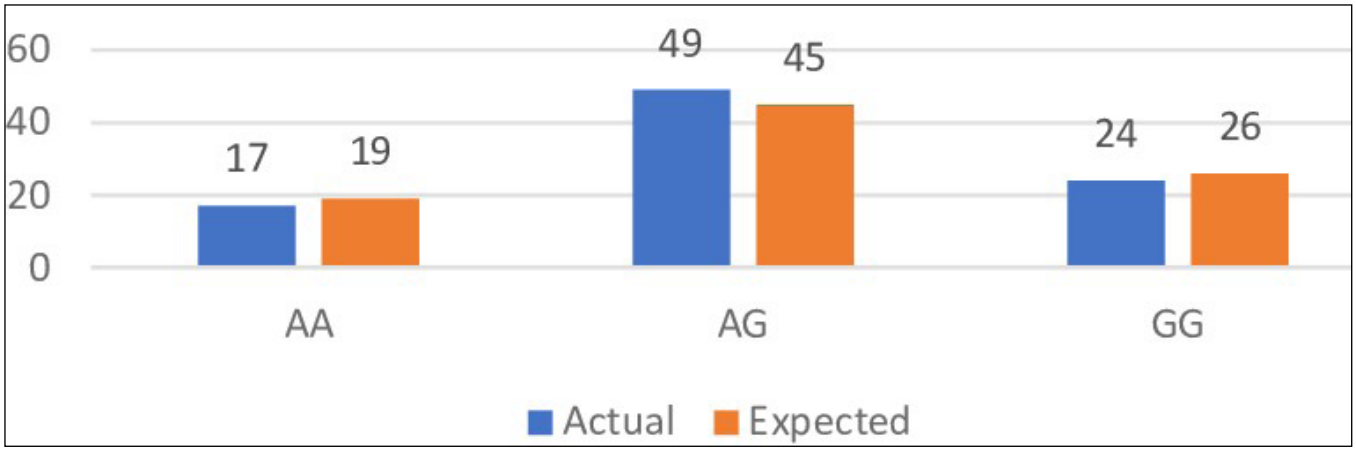

Fig. 4. Genotyping frequency of CYP11B2 Gene rs1799998 among Iraqi hypertensive patients taken valsartan 160 mg/day.

DISCUSSION

The finding of this study agrees with the study in Thailand population suggested that rs 1799998 is not associated with hypertension [11]. Also, based on the results of a meta-analysis, it appears that CYP11B2 polymorphism rs 1799998 in the RAAS have no significant effect on Bp salt sensitivity [12]. While the result of Iraqi study of Yarmouk teaching hospital in Baghdad who found that the 344G variant of CYP11B2 was not associated with hypertension in male subject they found that hypertension was associated with 344G alleleCYP11B2 Gene only in female [13]. This study disagrees with many studies of Han chines where there was a positive association between Aldosterone synthase gene CYP11B2 and high blood pressure [14]. A number of studies analyzed associations between rs1799998of CYP11B2 and hypertension with varying results [15]. Researchers found that CYP11B2 (rs179998) is significantly associated with longevity in Han Chinese participants. Japanese and European populations have also observed positive links between the rs1799998 polymorphism and HTN [16]. Another intriguing investigation found significant relationships between the CYP11B2 344G/A (rs179998) and essential HTN in South Indian Tamils (17). Niu et al. observed significant positive relationships between CYP11B2 gene polymorphism and the onset of hypertension in a study of Japanese populations [18]. Regarding rs 4539 the most frequent allele was T (62%) while the most frequent genotype was TC (60%), TT (32%), while c allele frequency was 38% and CC genotype frequency 8% with no significant difference P>0.05. The mean systolic BP and diastolic BP in homozygous TT carriers were 149 ±2.8 mmHg, and 90 ± 1.2 mmHg respectively. In homozygous CC carriers, the mean systolic BP and diastolic BP were 144 ± 4.7 and mmHg, 88± 2.2 mmHg respectively while in heterozygous TC carriers the systolic BP was 152±1.9 mmHg and Diastolic BP was 89±0.9 mmHg. There was no statistically significant effect of CYP11B2 rs 4539 on systolic and diastolic blood pressure. This study agrees with study of Hiromichi Tanahashi et al., in Japan Gifu university that indicated no association between rs 4539 and hypertension and rs4539 associated higher CYB11B2 gene expression [19]. Also, this study agrees with Chen et al., in south west Han chines population that indicated no association between rs 4539 in CYP 11B2 Gene and Essential hypertension [20]. Also, it agrees with study of CYP11B2 gen polymorphism in Pakistan 2023 that indicate no association between rs 4539 and hypertension [21], while this study disagree with other studies that indicate, *CYP11B2* (K173R) (rs 4539

Table 1. The sequencing and amplicon size of the PCR primers and annealing

Primer	Sequence	Amplicon	Annealing
CYP11B2 rs1799998	Inner forward CTTTATCTTATCGTGAGATGAGAGTGA 27	A-allele: 194 bp. G-allele: 233 bp. Two outer primers 370 bp.	57.5 °C
	Inner reverse AAATAAAGTCTATTAAAAGAATCCAAGTCC 30		
	Outer forward CAGCCAAAGGTAGATGAAGGA 21		
	Outer reverse TAACAACGTATCGAGATTCCTCAC 24		
CYP11B2 rs4539	Inner forward CGTTCTGCAGCACCTTCTGCC 21	C-allele 164 bp. T-allele 227 bp. Two outer primers 347 bp.	64 °C
	Inner reverse AGGGACTTCTCCCAGGCCCTTAA 23		
	Outer forward GATGCACTGCTGAGCACAAGGC 22		
	Outer reverse CTCTGCCCTGGCCTCTGTAGGAAT 24		

Table 2. Chemicals with their company and country of origin

No.	Chemical	Company and Origin
1	Agarose	MarLiJu (Korea)
2	Ethidium bromide	Intron (Korea)
3	Ladder	Bioneer (Korea)
4	Primers	Macrogen (Korea)
5	TBE buffer	Intron (Korea)

Table 3. The PCR thermocycler conditions

PCR step	Temp.	Time	Repeat
Initial denaturation	95°C	5min.	1
Denaturation	95°C	1min.	35cycle
Annealing	64°C/57.5°C*	1min.	
Extension	72°C	1min.	
Final extension	72°C	7min	1

* Annealing temp 64°C for rs4539 and Annealing temp 57.5°C for rs1799998.

Table 4. Genotype frequency of CYP11B2 gen polymorphism among Iraqi hypertensive patients who took valsartan 160 mg/day

CYP11B2	Genotype	Actual		Expected by Hardy-Weinberg law		P value
		Number	Frequency	Number	Frequency	
rs1799998	AA	17	0.19	19	0.21	0.8 (NS)
	AG	49	0.54	45	0.50	
	GG	24	0.27	26	0.29	
	Total	90	1.000	90	1.00	
	Allele					
	A	83	0.46	NA	NA	-
	G	97	0.54	NA	NA	-
	Total	180	1.00			
rs4539	TT	29	0.32	35	0.39	0.14 (NS)
	TC	54	0.60	42	0.47	
	CC	7	0.08	13	0.14	
	Total	90	1.00	90	1.00	
	Allele					
	T	112	0.62	NA	NA	
	C	68	0.38	NA	NA	
	Total	180	1.00			

in the exon3) hypertension was discovered to be linked to polymorphisms [22]. Other study in Indian patient indicates strong synergistic effect has existed among different genotypes of *CYP11B2* G344A, IC, and T173C (rs4539) polymorphisms with the haplotype (344A-Conv-T173) associated with a higher risk for essential hypertension progression

Table 5. The effect of CYP11B2 rs1799998 (A344>G) polymorphism on systolic and diastolic blood pressure in Iraqi hypertensive patients taken valsartan 160 mg/day

Genotype rs1799998	Systolic BP means	SE	P value	Diastolic BP mean	SE	P value
AA	152	3.2		91	1.7	
AG	150	2.2	0.12 (NS)	89	1	0.07
GG	144	2.8		86	1.1	

Table 6. The effect of CYP11B2 rs4539 (T2718C) polymorphism on systolic and diastolic blood pressure in Iraqi hypertensive patients taken valsartan 160 mg/day

Genotype rs4539	Systolic BP means	S E	P value	Diastolic BP mean	S E	P value
TT	149	2.8		90	1.2	
TC	152	1.9	0.14 (NS)	89	0.9	0.781
CC	144	4.7		88	2.2	

[23]. In meta-analysis study in China 2013, there was still not enough evidence to indicate the association between rs4539 T2718C with primary aldosteronism risk [24]. These variations are a reflection of the impact of demographic variables such as age and gender on populations that are geographically dispersed. Since the mechanism of the rs1799998, rs4539 variation in hypertension is unknown, large-scale studies are required to further understand the relationship between CYP11B2 polymorphism and the prevalence of hypertension.

CONCLUSIONS

Regarding rs4539 the most frequent allele was T (112, 62%) while the most frequent genotype was TC (54, 60 %). Regarding rs1799998 the most frequent allele was G (97, 54%) while the most frequent genotype was AG (49, 54 %). There was no significant difference between the actual and expected frequency distribution (P>0.05). There was no significant relationship between rs1799998 (A344>G) and rs4539 (T2718C) with systolic and diastolic blood pressure in Iraqi patient with essential hypertension.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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