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Polymorphism of Angiotensin Type 1 Receptor Gene (SNP rs5186 A1166C) Related with Hypertension Patients in Baghdad

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Abstract: This study was done to detect the contribution of *at1r* (A1166C) SNP rs5186 gene polymorphism in some Iraqi patients diagnosed with essential hypertension. This study has been started from October 2017 till the end of June 2018. Five ml of blood samples were collected from 100 subjects including 50 patients and 50 apparently healthy persons as a control group. DNA was extracted from all samples using DNA extraction kit (gyscn, geneaid) and PCR was performed to detect SNP (A1166C). The results showed that AA genotype frequency was higher in healthy people (68%) than in patients (42%) with highly significant differences, and AC frequency was higher in patients (50%) than in control (30%) with highly significant differences ($p<0.01$).And CC genotype was only 1(2%) in control, and 4(8%) in patients. The allele frequencies were as follow (A) allele were 0.83 in control and 0.67 in patients (P). Furthermore the (C) allele frequency was as follow, 0.17 in control and 0.33 in patients. We concluded from the current study that the Angiotensin type 1 Receptor (AT1R) A1166C gene polymorphism, may be associated with hypertension in some Iraqi patients .and the C allele may be considered as a risk factor for developing hypertension.

Keywords: Hypertension, Angiotensin Type 1 Receptor (AT1R) gene polymorphism, SNP.

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Introduction:

Hypertension can be defined as an elevation of blood pressure (BP) beyond 140/90 mm Hg, hypertension is strongly correlated with adverse consequence outcomes such as ischemic heart disease, stroke, heart failure, and end stage renal disease. The challenges of managing hypertension , controlling and preventing the development of these latter outcomes are unlikely to relent; the global overburden of hypertension is projected to increase by 60% to affect approximately 1.6 billion adults worldwide by 2025 (1).

Clinicians and physicians considered 140 mmHg as the maximum normal adult Systolic BP value, and 90 mmHg as the upper limit for normal Diastolic BP value, as suggested by the World Health Organization (WHO) (2).

The renin-angiotensin system is an important component of blood pressure regulation, playing an important roles in saltwater homeostasis and vascular tone, and was suspected to be involved in hypertension.(3).

The rennin-angiotensin-aldosterone system (RAAS) is one of the most important hormonal systems, monitors the functions of cardiovascular, renal, and adrenal glands by regulating blood pressure, sodium and potassium balance and fluid volume. Abnormal activity of

the RAAS result in the development of an array of cardiovascular diseases (CVD; hypertension, atherosclerosis, and left ventricular hypertrophy), cardiovascular events (stroke, myocardial infarction, and congestive heart failure), and renal disease (4).

Blood pressure regulation through the renin-angiotensin system (RAS) is intervened through the activation and inhibition of angiotensin II (AngII) from its precursor angiotensin I (AngI). Activation of AngII guides to vasoconstriction, leading to an increase in blood pressure, whereas AngII inhibition prevents vasoconstriction, causing a decrease in blood pressure. AngII in humans binds with two subtypes of angiotensin G protein-coupled receptors (GPCRs): AngII type 1 receptor (AT_1R) and AngII type 2 receptor (AT_2R). Almost all physiological and pathophysiological influences of AngII are mediated by AT_1R, while the role of AT_2R stays largely unknown. AT_1R receptor blockers (ARBs), or sartans, are non-peptide antagonists that plays on behalf of the RAS cascade to inhibit vasoconstriction, by that lowering blood pressure (5, 6).

AT1R gene plays an important role in the cardiovascular system, such as vasoconstriction, smooth muscle cell growth and cellular hypertrophy governed by major signaling mechanisms.AT1R A1166C gene polymorphism is associated with essential hypertension in many population (4, 6, 14)

The mechanism underlying rs5186-hypertension association is not clear since the SNP is placed within the 3′ untranslated region of *AGTR1*. It is possible that rs5186 is in linkage disequilibrium with other functional polymorphisms. This SNP, alternatively, may be involved in regulation of *AGTR1* expression. Despite the elusive role of the rs5186 SNP, the AGTR1 has been chosen as the potential treatment target for hypertension due to its evident relation with hypertension in certain population groups and effectiveness of its antagonists as antihypertensive therapy (6, 18).

This study was conducted to detect the contribution of *at1r* (A1166C) gene polymorphism in some Iraqi patients diagnosed with hypertension.

Materials and Methods:

Subjects:

Patients Group:

During the period of this study (October/2017 – June/2018), 50 Iraqi patients diagnosed with primary hypertension who attended Al-Yarmuk teaching hospital in Baghdad were registered. Their age ranged from 20 to 70 years. All patients were diagnosed to have primary hypertension.

Control Group:

Fifty apparently healthy people, aged between (20-70) were enrolled in this study.

DNA Extraction:

DNA was extracted by DNA extraction kit (gyscn , geneaid)from both patients and control group and the measurement of concentration and purity was done by the use of nanodrop (NAS99, BioAvans).

DNA amplification:

Genotyping of Angiotensin Type 1 Receptor (AT1R)A1166C:

One SNP A1166C have been genotyped in both cases and control in this study, The selected SNP was amplified by conventional polymerase chain reaction (PCR),using this specific primer ;Forward 5'-GCA GCA CTT CAC TAC CAA ATG GGC-3' and Reverse 5'-CAG GAC AAA AGC AGG CTA GGG AGA-3'(7).

Table (1): Primer reference

Primers for AT1R	Sequences 5´ → 3´	Primer reference
Forward	5'-GCA GCA CTT CAC TAC CAA ATG GGC-3'	Designed by Grzegorz Dzida *et al*.,2001
Reverse	5'-CAG GAC AAA AGC AGG CTA GGG AGA-3'	

Table (2): PCR reaction components for amplification of at1r gene

Component	Quantity (µl)	Concentration
Forward primer	1	10(pmol)
Reverse primer	1	10(pmol)
DNA template	5	25(ng/ µl)
D.W	13	-
Total volume	20	-

Table (3): PCR amplification program for at_1r gene.

Step	Temperature (°C)	Time min	No. of Cycles
Initial denaturation	94	5	1
Denaturation	94	1	35
Annealing	60	1	
Extension	72	1	
Final extension	72	5	1

After the amplification the DNA fragments were separated on 2% agarose gel and visualized on UV-transilluminator the studied fragment size was 255 bp, 231bp and 24 bp as shown in the figure 1and 2.

Enzymatic Digestion:

HaeIII Enzyme Digestion:

PCR product were subjected to RFLP analysis using restriction enzyme *HaeIII* (isolated from the *Haemophilus aegyptius* bacteria) as follow : when mutant allele (cytosine), digested with *HaeIII* that made two fragments ,whereas a wild allele (adenine) at nucleotide position 1166, do not have a cutting site (four bases: 5'GGCC3' and 3'CCGG5') for *HaeIII*, so that the PCR product was not cleaved into 2 fragments. The restriction digest products were visualized after electrophoresis on a 3% agarose gel and ethidium bromide staining (8).

Statistical analysis of data:

The Statistical Analysis System-SAS (2012) was used to identify the effect of different factors in study's parameters (percentage). The Chi-square test was used to determine significant differences between percentages in this study. Also the allele frequencies were calculated. Odds ratio were calculated for genotypes among control and patients. The confidence interval (CI) at 95% was done to

demonstrate the amount of uncertainty related with samples, the significance at value $p < 0.05$ (9).

Results and Discussion:

Age distribution:

One hundred individuals participated in the present study, including the following groups:

Group 1: This group included 50 samples of hypertensive patients. Their ages ranged from 20-70 years.

Group 2: This group included 50 samples of apparently healthy individuals. Their ages ranged from 20 to 70 year, with a mean of (45.51) year.

According to age results, 66% of apparently healthy control and 18% of hypertensive patients aged between 10-40 years old, 24% of apparently healthy control and 28% of hypertensive patients aged between 40-50 years. And 10% of apparently healthy control and 54% of hypertensive patients aged between 50-75 years old. Most of hypertensive patients were within the range 50-57 of age.

Table 1 Distribution of normotensive and hypertensive groups according to their age.

Age group (years)	Control		Patients	
	No.	%	No.	%
Less than 40	33	66	9	18
(40-50)	12	24	14	28
More than50	5	10	27	54
Total	50	100%	50	100%

Sex distribution:

In control group the AA genotype of AT1R gene was 23 in male, 11 in females with a highly significant differences ($p<0.01$). The AC genotype was 4 in male, 11 in female with a highly significant differences ($p<0.01$), and there was non-significant differences between male (1) and female (0) in CC genotype.

In patient group the AA genotype was 10 in male, 11 in female. Ac genotype was higher in female (19) than in male (6) with a highly significant differences ($p<0.01$). While the CC genotype was 0 in male, 4 in female with a significant differences ($p<0.01$).

Table 2 Distribution of sample study according to gender in AT1R gene in control and patient groups

AT1R gene	AA	AC	CC	Chi-Square (χ^2)
Control Group				
Male 28	23 (82.14%)	4 (14.29%)	1 (3.57%)	13.84 **
Female 22	11 (50.00%)	11 (50.00%)	0 (0.00%)	11.75 **
Chi-Square (χ^2)	9.62 **	10.06 **	1.074 NS	---
Patient Group				
Male 16	10 (62.50%)	6 (37.50%)	0 (0.00%)	12.94 **
Female 34	11 (32.35%)	19 (55.88%)	4 (11.76%)	10.37 **
Chi-Square (χ^2)	9.16 **	8.72 **	4.97 *	---

**** (P<0.01), NS: Non-Significant.**

Table 3 Distribution and Allele frequency to AT1R Polymorphism in healthy and patients

AT1R Polymorphism	Healthy (n=50) Frequency	Patient (n=50) Frequency	P- value	Odd ratio (95% CI)
AA	34 (68.00%)	21 (42.00%)	0.0068 **	1.374
AC	15 (30.00%)	25 (50.00%)	0.0096 **	1.266
CC	1 (2.00%)	4 (8.00%)	0.089 NS	0.327
Alleles freq.				
A	0.83	0.67	---	---
C	0.17	0.33	---	---

**** (P<0.01), NS: Non-Significant.**

The result in this study showed that AA frequency was higher in healthy people (68%) than in patients (42%) with highly significant differences. And AC frequency was higher in patients (50%) than in control (30%) with highly significant differences (P<0.01).

A significant association have been found in the AT1R genotypes (AC+CC) in essential hypertension (χ^2=22.48, p=0.0001). Patients with CC genotypes were at 2.4 times higher odds (p=0.0001) to develop essential hypertension than persons with AC and AA genotypes (10).

PCR-RFLP Analysis for *at1r* gene rs5186:

The SNP (rs5186) in *at1r* gene was detected as 255 bp band (figure 1), to diagnose the alleles of SNP (rs5186). PCR products were subjected to *HaeIII* restriction enzymes. The following fragment sizing patterns were detected by agarose gel electrophoresis:

Homozygous AA (255bp) was digested in two fragments (231bp, 24bp) (Figure 1, Lane 4, 5, 7, 8). Heterozygous AC, *HaeIII* restriction enzyme used to show three fragments in agarose gel electrophoresis (255 bp, 231 bp and 24 bp) (Figure 1, Lane 1, 2, 6).

Homozygous: CC (231) PCR fragments weren't digested. (Figure 2)

The 24bp was not clear in the figure (1).

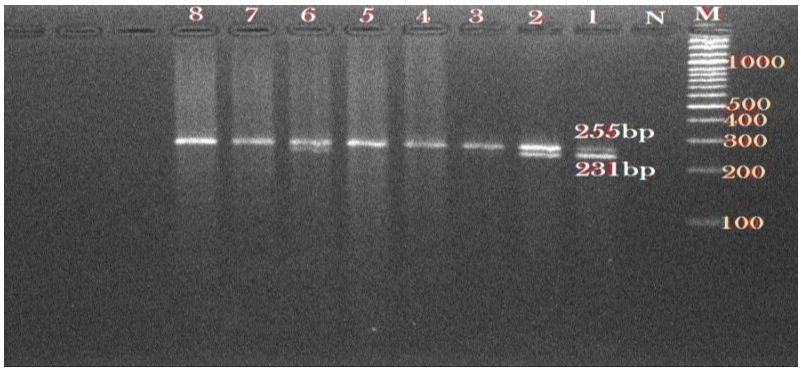

Figure (1): PCR Product for *at1*r Gene

A photograph of PCR product for *at1r* gene stained with EtBr in 3% agarose gel at 70 volt for 1 hour, 100 bp DNA ladder, showing AA and AC genotype.

Homozygous AA (255bp) was digested in two fragments (231bp, 24bp) (Figure 1, Lane 4, 5, 7, 8). Heterozygous AC, *HaeIII* restriction enzyme used to show three fragments in agarose gel electrophoresis (255 bp, 231 bp and 24 bp) (Figure 1, Lane 1, 2, 6).

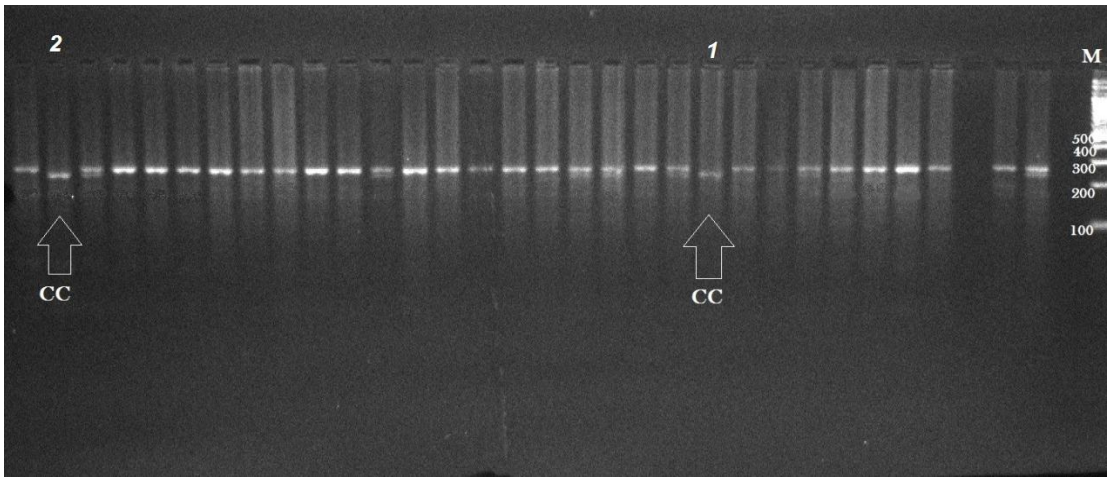

Figure (2): PCR Product for at1r Gene (Showing CC Genotype)
A photograph of PCR product for *at1r* gene stained with EtBr in 3% agarose gel at 70 volt for 1 hour, 100 bp DNA ladder 1 and 2 showing the CC genotype (231 bp).

Effect of *at1r* gene Mutation on Hypertension:

The genes of Renin Angiotensin Aldosterone System are the most likely sensible genes in essential hypertension. The major components of this system include angiotensin, renin, angiotensin II, angiotensin II type 1 receptor (AT1R), aldosterone, and so on is mainly distributed in heart and vascular smooth muscle. The vasoconstriction is produced after binding angiotensin II. In 1994, Bonnardeaux *et al* has detected five kinds of genetic polymorphisms, among which A1166C, T573C, and A1062G were the most frequently replaced, and the most frequently studied topics are on the association of A1166C polymorphism and essential hypertension. A number of studies indicated that A1166C polymorphism was highly associated with EH (11).

This study suggests that C allele may be a susceptible gene for EH. And is associated with the incidence of hypertension in this sample of Iraqi people. These results were concluded from numbers and proportions of three genotypes AA, AC, CC in patients and apparently healthy people.

After bonding to AT1R, Angiotensin II has been activated, causing vascular relaxation and the release of endothelin (ET). It also results in changes in the biological effects of water and salt metabolism, and cardiac and vascular wall fibrosis. It was reported that the levels of endothelin in carriers of CC genotype were higher than that of AA and AC, while polymorphism of AT1R A1166C participated in the hypertension incidence through increasing endothelin level (12).

Mehri *et al* conducted a case-control study of 142 hypertension cases and 191 controls, and have been

found that the risk of hypertension in the carriers of CC genotype was 3.45 times that of the AA genotype (13).

Jinmin *et al* has studied the distribution of AT1R gene A1166C polymorphism in a case-control study, which included 250 cases of hypertension and 250 cases of apparently healthy control. He found that the risk of developing hypertension in the carriers of AC+CC genotype was 2.4 times of AA genotype (14).

But, no association of A1166C polymorphism and hypertension was found in studies have been done in Nigeria and Jordan (15 and 16).

Junwang *et al* found that the AC+CC genotype carriers were 1.758 times more prostrate to suffer from essential hypertension than the AA genotype carriers (17).

The etiology of hypertension is much complex. It is caused by number of genes, environmental factors, and their interactions. The interaction means that when both factors are present, their true impact could be either larger than (synergistic) or less than (antagonism) the sum of individual impacts, should only one be present. Thus, understanding the interactions between genes and environmental factors plays a critical role in the control and prevention of hypertension. This would require the continuing challenge of identification and reasonable explanation of these relationship and interactions (17).

In conclusion, it have been concluded that the Angiotensin Type 1 Receptor (AT1R) A1166C gene polymorphism is linked to hypertensive patients within Iraqi population and the CC genotype maybe considered as a risk predictor for developing hypertension among individuals in Iraqi population.

References:

1. Kearney, P.M.; Whelton, M.; Reynolds, K. *et al*. (2005). Global burden of hypertension: analysis of worldwide data. Lancet;365:217–23.
2. Shrout, T.; Rudy, D.W. and Piascik, M.T. (2017). Hypertension update, JNC8 and beyond. Curr. Opin. Pharmacol, 33, 41–46.
3. Hall, J.E. and Guyton, A.C. (1990). Control of sodium excretion and arterial pressure by intrarenal mechanisms and the renin-angiotensin system. In: Laragh JH, Brenner BM, eds. Hypertension: Pathophysiology, Diagnosis and Management. New York, NY: Raven Press, Publishers:1105-1129.
4. Ferrario, C.M. and W.B. Strawn. (2006). "Role of the renin-angiotensin aldosterone system and pro inflammatory mediators in cardiovascular disease," American Journal of Cardiology, vol. 98, no. 1,pp. 121–128.
5. Emily, F.; Lavin**,** S.; Merus**,** G.; Ramirez V. and Vohra, R. (2017). Developing 3-D Molecular Models to Highlight the Angiotensin II Type 1 Receptor and Olmesartan Binding for Medical and Educational Applications. The FASEB Journal. 31(1).
6. Yako, Y.Y.; Balti, E.V.; Matsha, T.E.; Dzudie, A.; Kruger, D.; Sobngwi, E. *et al.* (2018). Genetic factors contributing to hypertension in African based populations: A systematic review and meta-analysis. South African Medical Research Council.
7. Dzida, G.; Sobstyl, J.; Pużniak, A.; Patrycja G.; Mosiewicz, J. and Hanzlik, J. (2001). Polymorphisms of angiotensin-converting enzyme hypertension in a Polish population. Med Sci Monit, 2001; 7(6): 1236-124.
8. Sipahi, T. (2012). Lack of Evidence for Contribution of eNOS, ACE and AT1R Gene Polymorphisms with Development of Ischemic Stroke in Turkish Subjects in Trakya Region. In Polymerase Chain Reaction. InTech.
9. Szumilas, M. (2010). Explaining odds ratios. Can Acad Child Adolesc Psychiatry, 19(3): 227-229.

10. Chandra, S.; Narang, R.; Sreenivas, V.; Bhatia, J.; Saluja, D. and Srivastava, K. (2014). Association of Angiotensin II Type 1 Receptor (A1166C) Gene Polymorphism and Its Increased Expression in Essential Hypertension: A Case-Control Study. Journal List,PLoS One, v.9(7).

11. Bonnardeaux, A.; Davies, E.; Jeunemaitre, X.; Féry, I.; Charru, A.; Clauser, E. *et al*. (1994). Angiotensin II type 1 receptor gene polymorphisms in human essential hypertension.Hypertension. 1994 Jul; 24(1):63-69.

12. Zhao, R.Z.; Shi, B. and Li, L. (2006). The study of relationship between polymorphism of angiotensin II type 1 Receptor (AT1R) gene and serum ang II and ET in patients of essential hypertension. Acta Academiae Med Zunyi; 29: 25–27.

13. Mehri, S.; Mahjoub, S.; Hammami, S. *et al*. (2012). Renin-angiotensin system polymorphisms in relation to hypertension status and obesity in a Tunisian population. Mol Biol Rep; 39:4059.

14. Jinmin, L.; Jianmin, L.; Shuqin, Z.; Xueqiu, L. and Shuyi, T. (2013). The polymorphism of angiotensin-receptor gene A1166C in familial hypertension and its distribution in the Han Yellow race of China. Saudi Med J; 34:1007–1012.

15. Kooffreh, M.E.; Anumudu, C.I.; Duke, R.; Okpako, E.C. and Kumar PL. (2013). Angiotensin II type 1 receptor A1166C gene polymorphism and essential hypertension in Calabar and Uyo cities, Nigeria. Indian J Hum Genet; 19:213–218 Uyo cities, Nigeria. Indian J Hum Genet; 19:213–218.

16. Shahin, D.S.; Irshaid, Y.M. and Saleh, A.A. (2014). The A(1166)C polymorphism of the AT1R gene is associated with an early onset of hypertension and high waist circumference in Jordanian males attending the Jordan University Hospital. Clin Exp Hypertens; 36:333–339.

17. Tong, J.; Wang, Y.; Yuan, J.; Yang, J.; Wang, Z.; Zheng, Y. *et al*. (2017). Effect of Interaction Between Noise and A1166C Site of AT1R Gene Polymorphism on Essential Hypertension in an Iron and Steel Enterprise Workers. Journal of Occupational and Environmental Medicine, 59(4): 412–416.

18. Liu, D.X.; Zhang, Y.Q.; Hu, B. *et al*. (2015). Association of AT1R polymorphism with hypertension risk: an update meta-analysis based on 28,952 subjects. J Renin Angiotensin Aldosterone Syst.; 16:898-9.