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Investigating The Single Nucleotide Polymorphism A1166C in Angiotensin II Type 1 Receptor Gene and Its Association with Hypertension in Patients in Syria

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ABSTRACT: *There are reported associations between a polymorphism of the angiotensin II type 1 receptor (AGTR.1/A1166C) gene and hypertension in some populations. To determine if the single nucleotide polymorphism (SNP) A1166C in AGTR.1 is associated with hypertension in Aleppo, we recruited twenty-eight patients with hypertension and thirty-five healthy subjects as the control group. The AGTR.1 polymorphism was assessed using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) based method. Results: The genotypic frequency of AA was 88.9%, that for AC was 11.1% and no CC type was detected. There was no significant difference in genotype and allele frequencies between hypertensive and non-hypertensive patients (p>0.05). Our results indicated that AGTR.1 A/C1166 polymorphism was not associated with hypertension in Syrian population.*

Keywords: *A1166C SNP, angiotensin receptor, gene polymorphism, hypertension, PCR-RFLP*

1. INTRODUCTION

Human essential hypertension is thought to result from the interaction of environmental and genetic factors, with approximately 30% of the interindividual variability in blood pressure being genetically determined [1]. The renin-angiotensin system is an important component of blood pressure regulation, playing roles in saltwater homeostasis and vascular tone [2], and has been suspected to be involved in hypertension. In the RAS Cascade, renin is released in response to certain stimuli into the circulation and acts on angiotensinogen. Renin cleaves angiotensinogen to produce angiotensin I (Ang I). Ang I has no biological action in itself, but is converted to angiotensin II (Ang II), by the angiotensin-converting enzyme (ACE), an enzyme present on the cell surface of many cells and particularly on vascular endothelial cells. Finally, Ang II will bind to specific cell surface angiotensin-receptors to elicit multiple actions. Two main cell surface receptors to Ang II have been identified: AGTR.1 and AGTR.2. Both the AGTR.1 and AGTR.2 have been cloned and belong to the superfamily of G protein-coupled receptors that contain seven transmembrane regions. The AGTR.1 mediates all of the classical actions of Ang II (vasoconstriction, sodium retention, cell growth and proliferation). AGTR.2 promote vasodilatation, cell differentiation, inhibition of cell growth and apoptosis, and may play a counterbalancing role to the effects of Ang II on AGTR.1 [3].The cellular effects of angiotensin II in adult humans are mainly mediated by the angiotensin type 1 receptor. The angiotensin II type 1 receptor (AGTR.1) gene has been cloned and mapped to the long arm of human chromosome 3 (3q21-q25) [4] While this gene polymorphism is associated with cardiac hypertrophy [5] and increased artery vasoconstriction [6, 7], it is difficult to interpret this association since the polymorphic variation is found in the non-coding region of the gene. Recently it has been suggested that the A1166C polymorphism may be involved in the regulation of the expression of AGTR.1 gene [8]. Interestingly a weak but significant linkage disequilibrium with a polymorphism in the promoter region of the AGTR.1 gene and AGTR.1/A1166C has also been reported [9].

The aim of this study was to determine the associations of the AGTR.1 A1166C marker with hypertension in Syria (Aleppo).

2. MATERIALS AND METHODS

The A1166C polymorphism in the 3` untranslated region of the AGTR.1 gene was determined using the method of Doria *et al.* [10]. The DNA amplification reaction was performed using PCR Thermal Cycler Dice™, Gradient Model (TaKaRa, Japan). All enzymes and chemicals used in this study were of molecular grade; Taq DNA polymerase (Thermo, USA) and *Dde*I (*HpyF3*I) restriction enzyme (Thermo, USA).

2.1. Patients

The study was performed with randomly recruited subjects from different areas in Aleppo, Syria. The hypertensive cohort was selected of at least 1 year duration and with antihypertensive treatment. Ages between 37-73 and a positive family history defined as the presence of at least one first-degree relative suffering from hypertension. None of them had diabetes mellitus, renal insufficiency or any primary causes and/or secondary hypertension. Although a larger number of individuals was initially screened, the inclusion criteria lead to a final number of 28 subjects (17/11 females/males) for the hypertensive cohort. The control group include 35 healthy patients (13/22 females/males). Ages between 35-60 and no family history of hypertension and cardiovascular disease in this group.

2.2. Genotyping

To determine the angiotensin II type 1 receptor genotype, genomic DNA was extracted from whole blood using the GF-1 Blood DNA Extraction Kit (Vivantis, Malaysia). All the polymorphism studies of AGTR.1 gene were conducted by polymerase chain reaction (PCR).

The primer design and reported polymorphisms were in the order of the genomic sequence of GenBank entry AF245699 (NCBI). The A1166C variant of the AGTR.1 gene was identified with primers: 5'-GCACCATGTTTTGAGGTTG -3' as the forward and 5'-CGACTACTGCTTAGCATA- 3' as the reverse primers. Briefly, for a 50 µL PCR, the reaction contained 200 ng genomic DNA, 200 µmol/l of each of dNTPs (dATP, dCTP, dGTP and dTTP), 250 ng of each primer, 3 mmol/l magnesium chloride and 1 U Taq DNA polymerase.

The polymerase chain reaction (PCR) process involved 5 minutes of denaturation at 94℃, followed by 35 cycles of 60 seconds denaturation at 94℃, 60 seconds annealing at 56.5℃ and 90 seconds extension at 72℃. The terminal extension was performed at 72 ℃ for 10 min. This resulted in a 546 bp PCR product. The PCR product was digested with the *Dde*I restriction enzyme. Digested products were separated by agarose gel electrophoresis on 2.5% agarose and visualized directly under UV light after staining with ethidium bromide 10 mg\ml (Vivantis, Malaysia). Undigested 546 bp fragment indicated the presence of the A allele and, appearance of two bands at 111 and 435 bp represented the C allele.

2.3. Statistical analysis

Statistical analyses were performed using the SPSS® Statistics 22 IBM®, included the Logistic Regression test for genotype and allele frequencies comparison. A level of $P<0.05$ was considered statistically significant.

3. RESULTATS

In vitro DNA amplification of the AGTR.1 gene using the specific primers resulted in a 546 bp DNA product (Fig. 1). The PCR products were digested with the *Dde*I restriction enzyme. The 1166C AGTR.1 allele contains a recognition site for the restriction endonuclease, *Dde*I, so that digestion of the PCR product with *Dde*I yields 435 bp and 111 bp fragments. The 1166A AGTR.1 allele does not contain a recognition site for the restriction endonuclease, *Dde*I, so that the 546 bp amplicon remains unaltered after incubation with *Dde*I [11, 12]. Thus, each of samples revealed one of the three different electrophoretic patterns (Fig. 2). Frequencies of the AA and AC/CC genotypes and alleles in the study population are presented in Table 1.

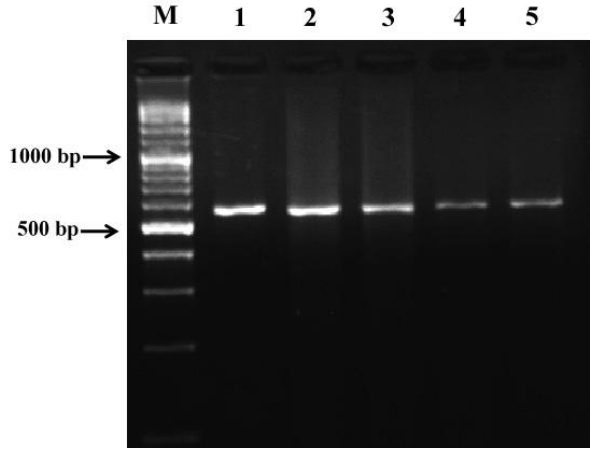

Fig. 1 Amplification of the 546 bp fragment of human AGTR.1 gene. M, VC 100 bp Plus DNA ladder; Lane 1 to lane 5 represent the 546 bp PCR product (amplicon).

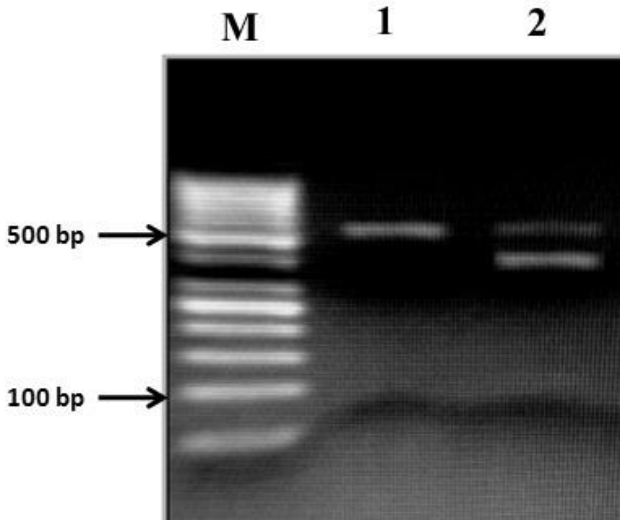

Fig.2 A1166C polymorphism of the AGTR.1 gene. M, 50 bp DNA ladder; Lane 1 546 bp band of the homozygous (AA); Lane 2, 546 bp, 435 bp and 111 bp bands of the heterozygous genotype (AC).

Table1. Genotype frequency distribution in hypertensive and control subjects for the A1166C polymorphism

	Total	Normotensive (%)	Hypertensive (%)
Genotype	N=63	n=35	n=28
AA	56	35 (62.5)	21 (37.5)
AC/CC	7	0 (0)	7 (100)
Allele	N= 126	n= 70	n=56
A	119	70 (58.8)	49 (41.2)
C	7	0 (0)	7 (100)

Given the small numbers of subjects carrying the C allele, subjects were divided into four groups, according to gender (male versus female) and genotype (AA versus AC/CC)

Values are counts, with the relative percentage of each group. SPSS® Statistics 22 was used for statistical analyses between hypertensive and control subjects (Table 2).

Table 2. Distribution of genotypes in the whole population

	Total	Normotensive (%)	Hypertensive (%)
Male	N=33	n=22	n=11
AA	30	22 (66.70)	8 (24.20)
AC/CC	3	0 (0.00)	3 (9.10)
Female	N= 30	n= 13	n=17
AA	26	13 (43.30)	13 (43.30)

AC/CC	4	0 (0.00)	4 (13.30)

We applied the Logistic Regression to test the association between Genotype, sex and hypertension (Table 3).

Table 3. Logistic Regression Model Estimating Effects of genotype and gender on the susceptibility to hypertension

Factor	B	SE	Wald	P-Value	OR
Genotype	21.717	14848.006	.000	.999	Exp (21.717)
gender	1.012	.569	3.156	.076	2.750
Constant	-1.012	.413	6.004	.014	.364

We did not find any association between Genotype and Hypertension (P>0.05) (Table 3).

We also applied the Logistic Regression to test the association between Allele, sex and hypertension. Values are counts, with the relative percentage of each group (Table 4).

Table 4. Distribution of Alleles in the whole population.

	Total	Normotensive (%)	Hypertensive (%)
Male	N=66	n=44	n=22
A	63	44 (66.7)	19 (28.8)
C	3	0 (0.00)	3 (4.5)

Female	N= 60	n= 26	n=34
A	56	26 (43.3)	30 (50.0)
C	4	0 (0.00)	4 (6.7)

Table 5. Logistic Regression Model Estimating Effects of allele C and gender on the susceptibility to hypertension

Factor	(B)	SE	Wald	P-Value	OR
Allele	21.552	14833.464	.000	.999	2291417502.962
gender	.983	.384	6.565	.010	2.672
Constant	-.840	.275	9.358	.002	.432

We did not find any association between allele and Hypertension (P>0.05), but we find there is a significantly differential between sex and hypertension (p<0.05) which mean that women is more susceptible to hypertension than men by nearly 2.5 times (p=0.010<0.05, OR=2.672) (Table 5).

In the present study, the prevalence of the AC genotype (25%) was observed within the hypertensive population, while in normotense subjects it was 0%. No homozygote CC genotype was observed (Table 6). Fig. 3 shows the genotype distribution in both hypertensive and normotensive subjects.

Table 6. Frequency of the genotypes of the angiotensin II type 1 receptor A1166C

		Normotensive (%)	Hypertensive (%)
Genotype	**AA**	35 (100)	21 (75)
	AC	0 (0.00)	7 (25)
	CC	0 (0.00)	0 (0.00)

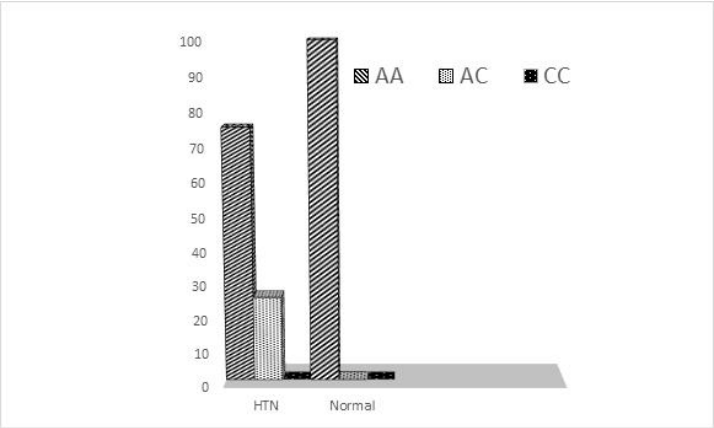

Fig 3. Genotype distribution according to hypertensive and normotensive subjects.

When all the subjects analyzed were re-arranged according to their genotype in the present study, the AGTR.1 genotype frequencies observed were (AA= 88.9%, AC= 11.1% and CC=0%) as shown in Fig. 4.

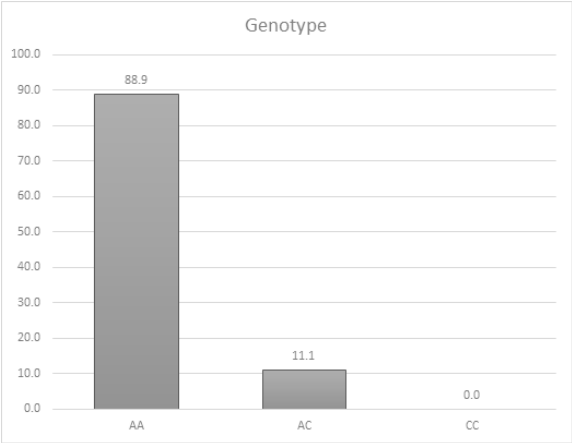

Fig 4. Genotype distribution in the study population

Frequency of the C1166 allele observed in this study (0.05) and Frequency of occurrence of the C1166 allele was higher among patients with hypertension (0.25) than in the control group (0).

4. DISCUSSION

Hypertension is one of the major risk factors for cardiovascular diseases. The prevalence of hypertension is increasing worldwide [13]. According to a recent study, One billion of the world's population has hypertension, resulting in four million deaths per year [14]. Data on the prevalence of hypertension in the Arab world are very limited. Only 13 studies were found in the literature from 10 Arab countries. The overall estimated prevalence of hypertension was 29.5% that indicates a similar prevalence of hypertension among Arabs compared to people from the USA (28%) and sub-Saharan African (27.6%) [15, 16]. The prevalence of hypertension was found to increase with age, occurring more frequently in Arab women [15, 17].

Human essential hypertension has been linked to genetic and environmental factors with genetic susceptibility being responsible for 30 to 50 % of the phenotype expression [18]. Many polymorphisms of the AGTR.1 gene have been reported, where an A to C substitution at position 1166 being the most widely studied [19-22]. Genetic studies have indicated that in many ethnic populations the substitution of cytosine for adenine at position 1166 was associated with susceptibility to essential hypertension [19, 22-25]. The present study is the first study performed in Syrian population of hypertensive patients. Restriction of the selection criteria for individuals with other causes of hypertension strengthened the correlation between AGTR.1 gene polymorphism and hypertension.

The A1166C polymorphism is located in a 3`-untranslated region (3`-UTR) of the gene and it has been proposed that the frequency of the C allele is increased in patients with hypertension. The potential role of the AGTR.1 gene in predisposition to hypertension is controversial. Since Bonnardeaux *et al*. [19] in 1994 reported higher prevalence of the C1166 allele among hypertensive than among normotensive subjects, a large number of studies have explored the relationship between AGTR.1 gene polymorphism and hypertension.

In the present study, we did not observe any significant difference between hypertensive and non-hypertensive patients in genotypes nor in their allele frequencies. The lack of association might be due to low sample size and type 2 error (low power to detect association). Previously, several studies have reported an association between A1166C polymorphism and hypertension, and higher frequencies of this SNP have been observed in hypertensive patients [25-27]. However, these results have not been well consistent. In some studies, subjects with CC genotype have been reported with lower blood pressure and cardiovascular risk [28, 29], while others have shown higher frequency of C allele or CC genotype among hypertensive subjects [25, 30, 31].

Furthermore, some other studies also failed to report any significant difference in genotype distribution between hypertensive and normotensive subjects or any significant association between A1166C polymorphism and hypertension [32-35].

5. CONCLUSION

In conclusion, investigation of the AGTR.1 A/C1166 polymorphism in a population in whom we have previously examined other major polymorphisms in Renin–Angiotensin System demonstrated the lack of an association with any clinical manifestations related to hypertension.

These observations are of interest for further studies in a larger population. Unfortunately, this study was designed to be cross-sectional with a restricted number of subjects. More studies with a prospective design and a larger number of subjects are needed to confirm our data. In addition, only a single gene effect was considered in our study. A complex genotype like that for hypertension would require the combined effects of more than two genes.

In addition, no CC genotype was detected in our study group so the sole effect of the C allele could not be determined. The role of other factors that can affect blood pressure, such as personal habits and environmental factors need to be more investigation to clarify this subject.

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