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Angiotensin II Receptor Gene A1166C Variant and Hypertension in Tunisian Population

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Abstract

Many genes have been proposed as candidate genes for hypertension. Among these genes, the angiotensin II type 1 receptor gene (AGTR1) has been investigated in the pathogenesis of hypertension, but studies have often generated controversial results. In this study, we analyzed the relationship between the A1166C variant of the AGTR1 and hypertension in a sample from the Tunisian population. Analysis of the AGTR1 genotypes was performed in 388 Tunisian patients with hypertension and 428 healthy subjects by polymerase chain reaction-restriction fragment length polymorphism. The results shows that the AGTR1 genotypes distribution and allele frequencies were not significantly different between the hypertensive and normotensive subjects ($p>0.05$). This polymorphism was not associated with hypertension (OR = 1.03, 95% CI [0.47-2.24]; p = 0.58) for AC and (OR = 1.19, 95% CI [0.65-2.19]; p = 0.83) for CC in comparison to the AA wild homozygous. After adjustment for the confounding factors of age, gender, body mass index, fasting glucose concentration, dyslipidemia and smoking, the OR for hypertension remained no significant (OR = 1.28, 95% CI [0.87-1.84]; p = 0.50) for CC vs AA. Furthermore, no relationship was found between clinical characteristics and AGTR1 genotypes. In the conclusion; our data suggested that the A1166C variant of the AGTR1 is not involved in the pathogenesis of hypertension in the Tunisian population.

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Keywords: Angiotensin II type 1 receptor gene; AGTR1; A1166C polymorphism; Hypertension

1. Introduction

Many factors may influence blood pressure (BP). The Renin angiotensin system (RAS) is one of factors that have an important role in controlling BP and vascular tone [1]. Consequently, genes that encode components of the RAS are thought to play a role in determining genetic susceptibility to hypertension (HTA).

Angiotensin II (Ang II), the active component of the RAS, binds to two major receptors, the Ang II type 1 receptor (AGTR1) and the Ang II type 2 receptor (AGTR2). The AGTR1 has multiple effects in several targets, including the blood vessels, kidney, brain and heart [2], leads to cascade of signaling pathways mediating vasoconstriction, inflammation and cell proliferation. These processes may induce cardiovascular diseases, such as atherosclerosis, ventricular hypertrophy and hypertension. Undoubtedly, hypertension is influenced by heredity and the AGTR1 variation has been suggested in this pathology. This gene has been found to be highly polymorphic in particular a SNP has been described in which there is an A/C transversion at position +1166. The AGTR1/A1166C polymorphism is located in the 3-UTR untranslated region of this gene. The increased frequency of the +1166C allele has been associated with hypertension [3], but the role of this polymorphism in the incidence of hypertension is controversial. However, despite confirmation in some studies [4-11] of the AGTR1/A1166C polymorphism involvement in the development of hypertension, other authors have reported divergent results [12-19] and have attributed these discrepancies to ethnic differences [18,19].

As the role of the AGTR1/A1166C polymorphism on the incidence of hypertension seems to be population-dependent, we examined in the present study, the relationship between the AGTR1/A1166C variant and hypertension in the Tunisian population.

2. Materials and methods

2.1 Study population

A total of 816 unrelated subjects living in the City of Tunis (Tunisia) were studied. They consisted of 388 hypertensive patients (HT) [128 men and 260 women; with a mean age 55.10 ± 9.9 years] and 428 normotensive subjects (NT) [232 men and 196 women; with mean age of 52.01 ± 13.10 years]. Controls were unrelated healthy volunteers, randomly collected among the families of hospital staff, without antihypertensive treatment, and their SBP and DBP were less than 140 and 90 mm Hg, respectively. Hypertension was defined according to World Health Organization criteria [20]. These criteria include a sitting systolic blood pressure (SBP) of ≥ 140 mm Hg or diastolic blood pressure (DBP) of ≥ 90 mm Hg on three occasions spanning 2 months from the first medical examination, without administration of antihypertensive drugs. Patients and controls were homogeneous Tunisian subjects, all were from North Tunisia. The ethical aspects of this study were approved by the local research committee (Rabta Hospital Ethics Committee, Tunis). For all participants, blood collection was performed after informed consent.

Body weight and height were measured on the subjects barefooted and lightly clothed. Body mass index (BMI; kg/m^2) was calculated and obesity was defined as BMI > 30 kg/m^2[21,22]. Diabetes mellitus was defined as hyperglycemia, requiring antidiabetic drugs fasting blood glucose over 7.0 mmol/L. Dyslipidemia was defined as a total cholesterol (TC) level > 6.47 mmol/L and/or triglyceride (TG) level > 2.26 mmol/L. Cigarette smoking was quantified based on daily consumption and duration of smoking.

2.2 Biochemical analysis

Blood samples were obtained after an overnight fast. fasting glucose, creatinine, uric acid concentrations, triglycerides (TG), total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), were determined by standardized enzymatic methods, using commercial kits (Roche Diagnostics, Mannheim, Germany), on a Hitachi 912 analyzer. LDL-C was calculated according to Friedwald's formula.

2.3 DNA analysis

Genomic DNA was extracted from peripheral blood leukocytes by phenol extraction [23]. Genotyping of the AGTR1/A1166C variant (rs5186) was determined by polymerase chain reaction-restriction fragment length polymorphism, followed by digestion with restriction enzyme DdeI [24,25]. PCR was performed in a reaction volume of 50 µl containing 200 ng of genomic DNA and primers, each at a final concentration of 250 ng (the forward: 5'GCACCATGTTTTGAGGTTG3' and the reverse: 5'CGACTACTGCTTAGCATA3'), 1.5 mmol/L $MgCl_2$, 200 □mol/L of each dNTPs (dATP, dCTP, dGTP and dTTP) and 1.5 U *Taq* polymerase (FERMENTAS FRANCE). DNA was amplified on a thermal cycler (BIOMETRA T1), according to the following protocol: initial denaturation at 94°C for 5 minutes and then 35 cycles of denaturation at 94° C for 30 s, annealing at 55° C for 30 s and extension at 72° C for 60 s, followed by a final extension step at 72° C for 10 minutes. Amplification products were then digested for 12 h at 37°C with the *Dde*I restriction enzyme (FERMENTAS FRANCE). Digestion products were separated in 3% agarose gel electrophoresis, stained with ethidium bromide and viewed under ultraviolet illumination. The different fragments obtained were 540 bp PCR in homozygous wild type AA, two fragments 430 and 110 pb in homozygous variant CC and all of these fragments for heterozygous.

2.4 Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS 11.5 for Windows; SPSS Inc., Chicago, IL, USA), and Epi Info (version 6.04a). The Student *t*-test was used for continuous variables, and the χ^2 test was used for categorical variables to test for statistical significance. We calculated unadjusted and multi-adjusted odds ratio (OR) together with their 95% approximate confidence intervals (95% CI) as estimators of the relative risk of hypertension for the AGTR1/A1166C (rs5186) genotypes. A binary logistic regression analysis was performed to determine the independent predictors for hypertension. We calculated the power in our samples to detect associations with different odds ratios, by using Quanto computer program [26]. A two-tailed *p*-value < 0.05 was considered statistically significant.

3. Results

Clinical characteristics of hypertensive and normotensive groups are given in Table 1. Compared to control subjects, hypertensive patients had a statistically higher mean of SBP, DBP, BMI and biological parameters: fasting glucose, TC, TG, LDL-C, creatinine and uric acid concentrations ($p<0.001$), but there were no significant differences between the two groups in HDL-C.

***Table 1*:** Baseline characteristics of the study population.

Variables	**NT (n=428)**	**HT (n=388)**	**p value**
Age (years)	52.01 ± 13.10	55.10 ± 9.90	0.08
BMI (Kg/m^2)	27.23 ± 4.87	31.13 ± 6.92	<0.001
SBP (mm Hg)	118.69 ± 10.69	151.57 ± 18.82	<0.001
DBP(mm Hg)	71.50 ± 6.86	87.63 ± 10.97	<0.001
Heart rate (beats/min)	78.79 ± 7.72	79.92 ± 7.51	0.07
Diabetes mellitus (%)	6.6	30.5	<0.001
Obesity (%)	27.4	50.8	<0.001
Dyslipidemia (%)	19.1	31.4	<0.001
Smokers (%)	35.3	20.1	<0.001
TC (mmol/L)	4.84 ± 0.95	5.31 ± 1.15	<0.001
TG (mmol/L)	1.43 ± 0.88	1.78 ± 1.02	<0.001
HDL-C (mmol/L)	1.27 ± 0.34	1.29 ± 0.34	0.25
LDL-C (mmol/L)	2.90 ± 0.80	3.18 ± 0.91	<0.001
Fasting glucose (mmol/L)	5.44 ± 1.83	6.60 ± 3.16	<0.001
Creatinine (μmol/L)	80.97 ± 13.34	87.42 ± 35.62	<0.001
Uric acid (μmol/L)	289.40 ± 83.3	317.96 ± 101.98	<0.001

BMI: body mass index; DBP: diastolic blood pressure; HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol; HT: hypertensive subjects; NT: normotensive subjects; SBP: systolic blood pressure; TC: total cholesterol; TG: triglycerides. Data values are means ± SD or % of patients, $p<0.05$ was considered significant.

The genotype frequencies were in agreement with those predicted by Hardy–Weinberg equilibrium conditions ($\chi^2 = 0.470$, $p = 0.711$). In total population, the distribution of genotypes in patients with hypertension was not significantly different from that in the control subjects (Table 2). There is also no statistical difference in the frequency of C risk allele (19% vs 20%, respectively) between the hypertensive group and controls. Subsequently, a stratified analysis of population by gender, because sex hormones might influence the mechanism of cardiovascular risk in patients, revealed also no significant genotypic and allelic differences between the two groups (Table 2).

***Table 2*:** Genotype distribution and allele frequencies of the AGTR1/A1166C genotype in the total population and according to gender.

	Genotype frequencies (%)			Allelic frequencies (%)	
	AA	**AC**	**CC**	**A**	**C**
All population					
NT (n= 428)	277 (64.7)	131 (30.6)	20 (4.7)	0.80	0.20
HT (n= 388)	254 (65.5)	119 (30.7)	15 (3.9)	0.81	0.19
		χ^2 = 0.32; p=0.84		χ^2 = 0.16; p=0.69	
Stratified by gender					
Men					
NT (n= 232)	147 (63.4)	72 (31)	13 (5.6)	0.79	0.21
HT (n= 128)	81 (63.3)	44 (34.4)	3 (2.3)	0.80	0.20
		χ^2 = 2.25; p=0.32		χ^2 = 0.26; p=0.61	
Women					
NT (n= 196)	130 (66.3)	59 (30.1)	7 (3.6)	0.81	0.19
HT (n= 260)	173 (66.5)	75 (28.8)	12 (4.6)	0.81	0.19
		χ^2 = 0.35; p=0.83		χ^2 = 0.03 p=0.87	

HT: hypertensive subjects; NT: normotensive subjects, n: subject number.

Values in parenthesis indicate percentage

In comparison to the AA homozygous, OR for hypertension was 1.03, 95% CI [0.47-2.24] p=0.58 for AC heterozygous and 1.19, 95% CI [0.65-2.19] p=0.83 for CC homozygous (Table3). When adjusting for confounding factors (age, sex, body mass index, fasting glucose concentration, dyslipidemia and smoking), in comparison to the AA homozygous, OR for hypertension remained no significant OR = 1.09, 95% CI [0.74-1.61]; p = 0.63 for AC heterozygous and 1.28, 95% CI [0.87-1.84] p=0.50 for CC homozygous (Table 3).

***Table 3*:** Odds ratio for AGTR1/A1166C gene polymorphism (crude and adjusted for confounding factors: age, sex, BMI, fasting glucose, dyslipidemia. and smoking) among the study subjects.

	Unadjusted OR 95% IC	P value	Adjusted OR 95% IC	P value
Genotypes				
AA	1		1	
AC	1.03 [0.47-2.24]	0.58	1.09 [0.74-1.61]	0.63
CC	1.19 [0.65-2.19]	0.83	1.28[0.87-1.84]	0.50
Alleles				
A			1	
C			1.24[0.65-1.47]	0.53

CI: confidence interval; OR: odds ratio.

Additionally, no relationship was found between clinical parameters and AGTR1/A1166C genotypes (Table 4).

***Table 4*:** Clinical characteristics of hypertensive patients and normotensive subjects according to AGTR1 A1166C genotype

Parameters	AA	AC	CC	p
Age (years)				
NT	54.48 ± 8.54	50.48 ± 8.29	49.10 ± 8.41	0.70
HT	55.85 ± 8.44	55.54 ± 8.36	54.20 ± 9.14	0.92
Gender (male/female)				
NT	277 (147/130)	131 (72/59)	20 (13/7)	0.57
HT	254 (81/173)	119 (44/75)	15 (3/12)	0.34
BMI (Kg/m^2)				
NT	27.13 ± 4.93	27.21 ± 4.57	28.82 ± 5.71	0.32
HT	31.37 ± 7.20	30.69 ± 6.40	30.65 ± 6.17	0.65
SBP (mm Hg)				
NT	118.73 ± 10.74	118.40 ± 10.64	119.95 ± 10.69	0.82
HT	151.21 ± 19.11	152.19 ± 18.58	152.80 ± 16.59	0.86
DBP (mm Hg)				
NT	71.47 ± 7.19	71.68 ± 6.24	70.75 ± 6.12	0.84
HT	88.04 ± 11.11	87.31 ± 10.97	83.33 ± 7.48	0.25
Heart rate (beats/min)				
NT	78.58 ± 7.59	78.82 ± 7.77	81.40 ± 8.99	0.29
HT	79.52 ± 7.59	81.05 ± 7.52	78.00 ± 4.95	0.11
Fastingglucose (mmol/L)				
NT	5.50 ± 2.10	5.21 ± 1.05	5.43 ± 1.05	0.25
HT	6.43 ± 2.99	6.77 ± 3.38	7.54 ± 3.88	0.32
Creatinine (µmol/L)				
NT	88.35 ± 13.61	81.68 ± 12.81	84.42 ± 13.26	0.32
HT	87.51 ± 38.71	88.57 ± 29.96	77.17 ± 18.12	0.50
Uric acid (µmol/L)				
NT	290.53 ± 87.10	286.37 ± 77.46	293.03 ± 65.86	0.87
HT	323.14 ± 98.53	311.66 ± 47.11	281.43 ± 106.32	0.22
TC (mmol/L)				
NT	4.86 ± 0.96	4.81 ± 0.93	4.58 ± 0.90	0.37
HT	5.33 ± 0.99	5.25 ± 0.94	5.12 ± 1.39	0.59
TG (mmol/L)				
NT	1.45 ± 0.90	1.40 ± 0.89	1.25 ± 0.55	0.59
HT	1.79 ± 0.97	1.78 ± 1.11	1.57 ± 0.73	0.72
HDL-C (mmol/L)				
NT	1.26 ± 0.33	1.24 ± 0.33	1.21 ± 0.28	0.67
HT	1.29 ± 0.33	1.29 ± 0.33	1.32 ± 0.28	0.96
LDL-C (mmol/L)				

NT	2.92 ± 0.82	2.90 ± 0.75	2.77 ± 0.90	0.71
HT	3.21 ± 0.93	3.13 ± 0.82	3.08 ± 1.16	0.67

BMI: body mass index; DBP: diastolic blood pressure; HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol; HT: hypertensive subjects; NT: normotensive subjects; SBP: systolic blood pressure; TC: total cholesterol; TG: triglycerides. Data are expressed as mean ± SD; <0.05 was considered significant.

4. Discussion

Many studies support the involvement of the SRA in the pathogenesis of hypertension. However, a clear relationship between the AGTR1 polymorphism and HTA is not consistently found in all ethnic groups.

Contrary to some studies, but in accordance with others, we report no association between the AGTR1/A1166C variant and HTA in our studied sample. Indeed, several reports did not show a significant association between AGTR1/A1166C and hypertension in Caucasian and Japanese populations [12-15,27-29]. However, other authors showed that A1166C genotype is strongly associated with increased risk of hypertension [4-11,30]. The same result was also reported by some studies in patients with severe early-onset disease [4] and in older and overweight patients with resistant essential hypertension [11]. This discrepancy may be due to several factors mainly ethnicity and selection criteria of patients as it was reported by some studies in subjects with long-term antihypertensive treatment or with a family history of arterial hypertension [3,9,13]. These contrasting findings may reflect different origins of hypertension and the genetic heterogeneity between different populations.

Since hypertension is a complex disease with many interacting parameters, we tested the implication of several known risk factors as (age, sex, BMI, fasting glucose, dyslipidemia, and smoking)but no significant difference was found and no relationship between AGTR1/A1166C genotypes and clinical parameters were obtained in our studied sample.

Furthermore, we observed that the prevalence of the C allele (19%) in our study was lower than to that reported in Caucasian subjects (22% - 36%) [12,13], but higher than in Asian (around 5%) and African populations (2%) [14]. Moreover, Liu *et al*. [31] studied three genetically different ethnic groups (Han, Tibetan and Yi populations) from the Chinese population and found that the A1166 allele frequency was different in these groups and only in Tibetan males, the A1166 allele may be a predisposing factor for essential hypertension. In support of this hypothesis, previous reports in which the genetic difference of Southern and Northern Chinese populations was considered [32,33], because many other factors influenced the relationship between the AGTR1 polymorphism and pathological phenotype, such as differences in lifestyle and eating habits between Southern and Northern Chinese. Thus, the discrepancy in genetic background could affect the predisposition to hypertension.

However, because data on the function of the AGTR1/A1166C variant are limited, the mechanism responsible for the association of hypertension with the A1166C polymorphism has remained largely unclear. The polymorphism is in a non-coding region of the gene, and therefore the amino acid sequence of the receptor is

not affected but it might alter mRNA stability and transcription. In support of this hypothesis, Spielman et al. [34] suggested that differences in SNP allele frequency among ethnic groups explain differences in gene expression. In this way, microRNAs (miRNAs) have been implicated in the control of various biological and pathological processes, as cardiovascular disease [35-38]. Interestingly, Ceolotto et al. [39] have reported that the 1166A/C is recognized by a specific miR-155, which is base-pairing complementary with the 1166A allele but not with the mutant 1166C. In this same study [39], authors have shown that AGTR1 protein expression was significantly increased in the mutant CC group and showed that 1166C polymorphism and AGTR1 protein expression may have a role in the regulation of BP.

Our data report lack of association between the AGTR1/A1166C variant and hypertension, but we must acknowledge certain limitation because our study was conducted with a limited sample size that might lead to a low statistical power that requires a sample size 2163 patients and 2184 controls to reach a power of 80% at a p=0.05.

5. Conclusion

In this study, our findings report a lack of association of the AGTR1 A1166C polymorphism with hypertension in Tunisian population and suggest that the role of this polymorphism on the incidence of HTA may be different among ethnic groups with differences in genetic background, lifestyle and eating habits.

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Conflict of interest

None declared.

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