

Original Article

Lack of association of the A1166C polymorphism in the angiotensin II type 1 receptor (ATR1) gene and essential hypertension in the South West Region of Cameroon

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Abstract: There has been inconsistent association of the angiotensin II type 1 receptor (ATR1) gene polymorphism and hypertension among various populations in different countries. With respect to these controversial reports, this study was designed to investigate if substitution of A for C at position 1166 (A1166C) in the ATR1 gene could be at the basis of hypertension in the Cameroonian population of the South West Region (SWR). Analysis of anthropometric data revealed that systolic and diastolic blood pressures as well as age could be classified as independent risk factors of essential hypertension. Eighty-six subjects (36 hypertensive and 50 normotensive subjects) were recruited from SWR and genotyped for A1166C polymorphism of ATR1 gene by restriction fragment length polymorphism. Genotype distribution was found to be 91.7% AA, 2.8% AC, and 5.6% CC for hypertensive subjects and 86% AA, 6.0% AC and 8.0% CC for normotensive subjects. Allele distribution was 93% A, 7% C for hypertensive subjects as against 89% A and 11% C for normotensive subjects. Analysis of results indicated that no significant differences in ATR1 genotype distribution ($P > 0.05$) and allele frequencies ($P > 0.05$) exist between hypertensive and normotensive subjects in the studied population. Also there was no significant difference in genotype distribution with sex. The study therefore concludes that there is lack of association between variants of the ATR1 gene and hypertension in Cameroonians of the SWR.

Keywords: Hypertension, risk factors, ATR1, genetic polymorphism

Introduction

Traditionally in Africa, communicable diseases accounted for the greatest burden of morbidity and mortality [1]. This burden is fast shifting towards chronic non-communicable diseases, and by extension cardiovascular diseases. This phenomenon constitutes what is known as “double burden of disease” [2]. Whereas hypertension was almost non-existent in African societies in the first half of the twentieth century, estimates now show that in some settings in Africa more than 40 percent of adults have hypertension [3]. There were approximately 80 million adults with hypertension in sub-Saharan Africa in 2000 and projections based on current epidemiological data suggest that this figure will rise to 150 million by 2025 [4]. Com-

plications of hypertension include neurological disorders, renal impairment [5], hypertensive retinopathy [6], stroke and heart failure [7, 8]. These trends have been strongly linked with both genetic as well as environmental factors (changes in individual and societal lifestyle such as an increase in tobacco use, excessive alcohol consumption, reduced physical activity and adoption of “Western” diets that are high in salt, refined sugar and unhealthy fats and oils) [9].

Studies have shown that about 50% of essential hypertension (EH) is hereditary [10]. Over the past 10 years, many genetic loci have been found to be associated with hypertension through candidate gene studies. Identification of these hypertension susceptible genes is

potentially useful to elucidate the complex genetic mechanisms of the disease. Recently single nucleotide polymorphisms (SNPs) in some candidate genes have been proven to confer susceptibility to the disease in some European and Asian countries but not others [11-13].

Angiotensin II receptors, which mediate the vaso-constrictive and salt-conserving actions of the renin-angiotensin system, represent interesting candidate genes for essential hypertension. A single nucleotide polymorphism (SNP) has been described in which there is either an adenine (A) or a cytosine (C) in position 1166 in the 3' untranslated region of the Angiotensin II type 1 receptor (ATR1) [14]. The A allele produces the fragment that lacks the restriction site for the Ddel enzyme but in the presence of cytosine (C allele), a restriction site is created for the enzyme. The physiological significance of this A1166C polymorphism is uncertain because of its location in an untranslated region. However, the polymorphism is associated with EH in the Caucasians and Serbian population [15, 16]; Han population in China [17]; France and Canada [18, 19]. Also, the polymorphism constitutes a high risk for cardiovascular diseases in San Luis (Argentina) [20]. On the other hand, studies undertaken in populations in Spain [21]; Japan [22] and Nigeria [23] have failed to show such association to A1166C polymorphism. The involvement of the ATR1 gene polymorphism in hypertension therefore seems to depend on the ethnic group and/or geographical location. No data have been reported thus far on the possible influence of A1166C polymorphism of the ATR1 gene on EH in Cameroon. In this study we sought to find out the risk of the A1166C polymorphism in the ATR1 gene in causing EH in South West Region (SWR) of Cameroon.

Materials and methods

Study population

The study group comprised of 50 normotensive controls and 36 hypertensive patients. The hypertensive patients were recruited from the diabetic and hypertension clinic of the Buea Regional Hospital of the SWR of Cameroon. Normotensive subjects were recruited from the outpatient department of the same hospital. These subjects had a SBP of less than 140

mmHg and a DBP of less than 90 mmHg and no history of EH in their first-degree relatives [22, 24]. Hypertensive subjects were defined as those having a systolic blood pressure (SBP) of 140 mmHg or greater and a diastolic blood pressure (DBP) of 90 mmHg or greater. Subjects who were already placed on hypertensive medication were also categorized as hypertensive. All the hypertensive patients were required to be free of secondary hypertension or diabetes mellitus, and to have been diagnosed with hypertension before the age of 60 years. All subjects gave their consent and ethical clearance for this work was obtained from the Faculty of Health Science Ethical Research Committee of the University of Buea.

Collection of anthropometric and biochemical data

A structured questionnaire was used for data collection on anthropometric variables (height, weight, and sex), family history and complications of hypertension. Body mass index (BMI) was calculated as weight, divided by height squared (kg/m^2). Resting blood pressure was measured in the right upper arm of subjects using a cardiocheck PA analyser (California, USA). Fasting Blood Sugar (FBG) was measured using the OneTouch UltraSoft lipid profile strip (Hanover, Germany) and analyzer.

Extraction of genomic DNA

DNA was manually extracted from blood using the phenol chloroform method [25]. Briefly, 5.0 mL of blood was centrifuged at 2,000 rpm for 10 min, the leukocyte layer (buffy coat) and the red blood cells (RBC) were mixed. Red blood cells were lysed with 10 mL of lysis solution (2 M Tris pH 7.6, 1 M MgCl_2 , 3 M NaCl) and the tubes centrifuged at 2,000 rpm for 10 min. The supernatant (lysed red blood cells) was aspirated and the pellet (white blood cells) resuspended in 5 mL white blood cell lysis solution (2 M Tris pH 7.6, 0.4 M EDTA pH 8, 3 M NaCl) supplemented with 0.25 mg/ml proteinase K. DNA was extracted from white blood cells using phenol chloroform mixture and precipitated with 10 mL of absolute isopropanol in the presence of 60 mM NaCl and washed in 3 mL of 70% ethanol in 0.1 M TE. DNA was then air-dried and resuspended in 0.5 mL of 0.1 M TE buffer and thereafter incubated overnight at 37°C with gentle rotation.

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Table 1. Anthropometric data of subjects

	N	Mean Age (years)	Mean BMI (Kg/M ²)	Mean SBP	Mean DBP
Hypertensive	36	62.14 ± 10.03	28.58 ± 5.22	160.44 ± 18.70	91.17 ± 15.16
Normotensive	50	50.26 ± 13.09	27.25 ± 6.80	123.2 ± 13.82	77.18 ± 11.76
P		0.000	0.912	0.00	0.00

Values are expressed as mean ± SD. SBP (systolic blood pressure); DBP (diastolic blood pressure); BMI (body mass index). SBP, DBP and BMI were compared by ANCOVA while Age was compared by Student t-test.

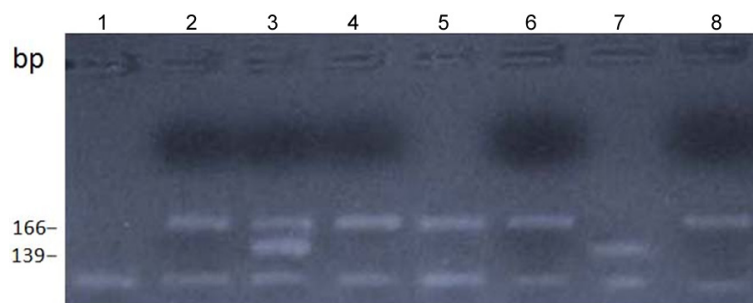


Figure 1. Dde I endonuclease digestion of PCR products. Genotypes AA: lanes 1, 3, 4, 5 and 7; Genotype CC: lane 6; and genotype AC: lane 2; Lane 8: negative control.

Amplification of the AT1R gene by polymerase chain reaction (PCR)

The PCR was carried out in a 25 µl reaction mixture comprised of 0.2 µg genomic DNA, 10 µM sense primer (5'-ATA ATG TAA GCT CAT CCA CCA AGA AG-3'), 10 µM antisense primer (5'-TCT CCT TCA ATT CTG AAA AGT ACT TAA-3'), 12.5 µl of PCR master mix containing 0.4 mmol/L of each dNTP (dATP, dCTP, dGTP and dTTP), 2.0 mmol/L MgCl₂, 1X Taq buffer and 0.05 U/µl Taq DNA polymerase. The pre denaturation step was carried out for 3 min. at 94°C. This was followed by a denaturation step at 94°C for 45 sec., annealing at 50°C for 45 sec. and polymerization at 72°C for 45 sec. with a final extension at 72°C for 5 min. Thirty cycles of PCR amplification was carried out in a PTC-200 MJ Research Peltier Thermal Cycler (Minesota, USA).

Restriction digestion of AT1R gene PCR products

One microgram of PCR product was diluted in 18 µl of nuclease free water, 2 µl of 10X Dde I buffer (Fermentas, Germany) and 10 U/µl of Dde I endonuclease was added. The reaction mixture was incubated overnight at 37°C and digests were separated on a 3% agarose gel

stained with ethidium bromide and visualized under UV light.

Statistical analyses

All the statistical analyses were carried out using SPSS (Chicago, IL) software version 14.0 for Microsoft Windows. Continuous variables were compared between the groups using two-tailed student t-test. Allelic frequencies were calculated by gene-counting method and the genotype distribution with Hardy-Weinberg expectations by a chi-squared test. A level of P < 0.05 was considered statistically significant.

Results

Characteristics of the study subjects

The distributions of sex, age, BMI, SBP and DBP for normotensive and hypertensive subjects are summarized in **Table 1**. There were significant differences in both SBP and DBP between normotensive and hypertensive subjects after adjustment for sex, age, and BMI (P < 0.001). There was also significant difference in age after adjusting for SBP, DBP, BMI and sex (P < 0.001) between the groups. No significant differences in BMI and sex were observed (P > 0.05) in both cases.

Genotype distributions and allele frequencies

Restriction digest of PCR products revealed three genotypes: AA, AC and CC (**Figure 1**).

The genotype distributions of the A1166C polymorphism were: 91.66% AA, 2.77% AC, and 5.55% CC in hypertensive and 86% AA, 6% AC, and 8% CC in normotensive subjects. The frequency of the A allele was 93% in hypertensives

Table 2. Genotype distribution and allele frequency between normotensive and hypertensive subjects

Group	N	Genotype frequency			Allele frequency	
		AA	AC	CC	A	C
HYP	36	33 (91.7%)	1 (2.8%)	2 (5.6%)	67 (93.0%)	5 (7.0%)
NOR	50	43 (86.0%)	3 (6.0%)	4 (8.0%)	89 (89.0%)	11 (11.0%)
		$\chi^2 = 0.383$ $P = 0.697$			$\chi^2 = 0.361$ $P = 0.602$	

Table 3. Genotype and allele distribution with respect to sex

Sex				Genotype			Allele	
				A	C	AA	AC	CC
Male	Control	n	15	1	3	31	7	
		%	78.94	5.26	15.78	81.57	18.43	
	Hypertensive	n	11	0	1	22	2	
		%	91.67	0.00	8.33	91.67	8.33	
	Female	n	28	2	1	58	4	
		%	90.32	6.45	3.23	93.55	6.45	
Female	Control	n	28	2	1	58	4	
		%	90.32	6.45	3.23	93.55	6.45	
	Hypertensive	n	22	1	1	45	3	
		%	91.66	4.17	4.17	93.75	6.25	
				$\chi^2 = 2.76652$ $P = 0.2507$			$\chi^2 = 3.1331$ $P = 0.0767$	

and 89% in normotensives while the C allele had a frequency of 7% in hypertensive and 11% in normotensive subjects (**Table 2**). The observed genotype frequencies were in agreement with those predicted by Hardy-Weinberg equilibrium. The genotype distribution and the allele distribution of the A1166C variants of ATR1 gene polymorphisms in hypertensive subjects were compared to those in normotensive subjects using the Pearson Chi-Square Test. No significant differences in the genotype distribution ($P = 0.697$, $\chi^2 = 0.383$) and allele frequency ($P = 0.602$, $\chi^2 = 0.361$) were observed between hypertensive and normotensive subjects (**Table 2**). Also there was no significant difference in genotype distribution with respect to sex (**Table 3**).

Discussion

Studies on A1166C polymorphism on the ATR1 gene still shows a wide variation in both genotype and allele frequency in different ethnic groups thereby suggesting that the involvement of this gene polymorphism in hypertension may be dependent on ethnic group. Our aim was to investigate if there is a possible association between this gene polymorphism and hypertension in Cameroonians of the South West Region.

Anthropometric factors considered to be risk factors of hypertension were age, SBP and DBP since the mean levels of each of these parameters were significantly higher in hypertensive than normotensive subjects (**Table 1**). The restriction pattern of the ATR1 gene revealed the presence of all the three possible genotypes in both study groups: the homozygous AA genotype appeared as a single uncut band of 166 bp while the homozygous CC was a single restricted band of 139 bp and the heterozygous AC genotype had two bands of 166 and 139 bp (**Figure 1**). The smaller restricted fragment had a size of 27 Bp and thus embedded in the primer band since they are of the same size. These results are consistent with those obtained by genotyping variants of the A1166C poly-

morphism in three ethnic groups (Han, Tibetan, and Yi) in China and where the three genotypes AA, AC and CC were obtained [24]. However studies carried out in Calabar and Uyo cities (Nigeria) failed to report the CC genotype [23] thereby suggesting that allelic frequency varies with ethnicity.

In our study, comparison of genotype distribution and allele frequencies between hypertensive and normotensive subjects did not reveal any statistical significant differences. For genotype distribution amongst the hypertensive subjects, 91.7% were of the homozygous AA genotype, 2.8% had the heterozygous AC genotype while 5.6% were homozygous for the CC genotype (**Table 2**). These results are similar to that of the control group where 86% were homozygous for AA, 6.0% heterozygous (AC) and 8% were homozygous (CC). Evidently, allele frequencies between the two study groups were similar: 93% of hypertensive patients had the A allele and 7% the C allele while 89% of the normotensive subjects had the A allele and 11% the C allele. These results suggest that there may be no relationship between ATR1 polymorphism and hypertension in the studied population. These results are similar to those obtained in different populations in Japan, [22] Ghana [26] and Nigeria [23] where there was

no significant difference in genotype or allele distribution between hypertensive patients and control subjects. On the other hand, these results are inconsistent with those obtained in France [18] and China [17] where there was a prevalence of the C allele in hypertensive subjects than in controls as well as in Egypt where the A allele constituted a predisposing factor for EH in Egyptians [27]. Although there was no significant difference in allele distribution in this study, it was observed that the A allele was more predominant in both patients and control subjects than the C allele. It would appear that the C allele is less frequent in Africans (Cameroonians, Nigerians and Ghanians) than in Caucasians.

Our study also showed no significant difference ($P > 0.05$) in both genotype and allele frequency between males and females (**Table 3**). In both healthy and hypertensive males and females, the AA genotype was preponderant (over 78%) as well as the A-allele frequency (over 81%) thereby suggesting no sex-related differences in ATR1 polymorphism and EH.

It can therefore be concluded that no significant difference in both genotype distribution and allele frequency exist between normotensive and hypertensive subjects, implying a lack of association between variants of the A1166C polymorphism in the ATR1 gene and EH in patients of the SWR of Cameroon. The identification of this polymorphism in this subset of the Cameroonian population as a health risk factor will not help people who are predisposed to EH in taking adequate health decisions in order to prevent the disease.

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Disclosure of conflict of interest

None.

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