

# Angiotensin II type 1 receptor A1166C gene polymorphism and essential hypertension in Calabar and Uyo cities, Nigeria

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**OBJECTIVES:** The angiotensin II protein is a vasoconstrictor that exerts most of its influence through the angiotensin II type 1 receptor (AT1R). Inconsistent association between the A1166C polymorphism of the AT1R gene and hypertension has been reported among various populations but not among the peoples of Calabar and Uyo. This study was designed to determine the frequency of the A1166C polymorphism of the AT1R gene and its association with hypertension in a sample population of Calabar and Uyo.

**MATERIALS AND METHODS:** A population-based case control design consisting of total of 1224 participants, 612 each of patients and controls were randomly recruited from hypertension clinics and the general population. Genotyping of the A1166C allele of the AT1R gene to identify variants was performed using polymerase chain reaction and restriction enzyme digestion. Multiple regressions were applied to test whether the A1166 genotypes were predictors of hypertension.

**RESULTS:** 99% of the study population had the wild type AA genotype, and 1% was AC heterozygous carriers of the A1166C polymorphism.

**CONCLUSION:** The A1166C polymorphism was not a predictor of hypertension in the sample population of Calabar and Uyo.

**Key words:** A1166C, hypertension, polymorphism

vasoconstrictor that exerts most of its influence through the angiotensin II type 1 receptor (AT1R). AT1R is a membrane bound G protein coupled-receptor that mediates the effects of angiotensin II.<sup>[1]</sup> The highly polymorphic human AT1R gene is 55 kb long having five exons and four introns, A1166C polymorphism is a single base substitution of adenine for cytosine at position 1166 in the 3' untranslated regions of the gene located on chromosome 13. The A allele produces the fragment that lacks the restriction site for the Dde1 enzyme but in the presence of cytosine, there is a restriction site for the enzyme (C allele). The physiological significance of the polymorphism is uncertain because data on the function of the AT1R polymorphism is limited. Thus, the mechanism responsible for the association of hypertension status with A1166C polymorphism has remained largely unknown, and the amino acid sequence of the receptor is not altered. It is however, thought to affect mRNA stability and transcription and is in linkage disequilibrium with some other polymorphism. The A1166C polymorphism is associated with prevalent hypertension, increased aortic stiffness.<sup>[4-6]</sup> This polymorphism has also been associated with other diseases such as left ventricular hypertrophy<sup>[7]</sup> pregnancy induced hypertension; early coronary disease and excessive vasoconstriction.<sup>[8-10]</sup> Stankovic *et al.*,<sup>[2]</sup> reported a significant association between this polymorphism and hypertension in males but not females. The frequency of the C1166 allele was high among hypertensives.<sup>[11,12]</sup> Some other studies have also reported a negative association between the

## Introduction

The angiotensin II protein is a well-known

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A1166C polymorphism and hypertension.<sup>[13,14]</sup> These variations were attributed to ethnic differences in the various populations.<sup>[14,15]</sup>

There are no published data on these polymorphisms in the ethnic populations in Nigeria. This study was carried out to determine if there is an association between A1166C polymorphism and hypertension in a sample population of Calabar and Uyo cities.

## Materials and Methods

### Population

A total sample population of 1,224 adult men and women from different ethnic groups was included in this study. Of this number, 612 were patients attending the hypertension clinics in the University of Calabar Teaching Hospital, Calabar, the University of Uyo Teaching Hospital, Uyo. The other 612 were individuals whose blood pressure was below 140/90 mmHg, who were also not taking hypertensive drugs and not below the age of twenty from the same population. These individuals served as the control group.

### Inclusion criteria

All patients were individuals whose BP were consistently above 140/90 mmHg or were taking hypertensive medications. Controls were individuals whose BP were consistently below 140/90 mmHg and were not taking hypertensive medications.

### Exclusion criteria

Females in the population using oral contraceptives were excluded from the study population.

### Collection of samples

Blood was obtained from thumb pricks and blotted onto a filter (Whatman, no 3) paper, allowed to dry at room temperature and preserved in plastic bags prior to DNA extraction.

### Ethical approval

Subjects included in the study gave informed consent, and ethical approval for the study was obtained from the joint University of Ibadan/University College Hospital

ethical review committee and each of the health establishment concerned-University Teaching Hospital Calabar, University of Uyo Teaching Hospital and the General Hospital, Calabar.

### Filter paper extraction of DNA

This was done according to Bereczky *et al.*<sup>[15]</sup> Pieces<sup>[1,2]</sup> of the filter paper about 5 mm in diameter were cut using a sterile blade for each samples. These pieces were placed in an eppendorf tube, soaked in 65 µl of T.E buffer. The tube was incubated at 50°C for 15 min in a water bath. The pieces were pressed gently at the bottom of the tube several times using a new pipette tip for each sample. The eppendorf tubes were heated again for 15 min at 97°C to elute the DNA. The liquid condensing on the lid and the walls of the tube was removed by a short centrifugation (2-3 s). The DNA extract that is the supernatant was kept at -20°C before use.

The angiotensin 11 type 1 receptor gene requires Polymerase Chain Reaction/Restriction Fragment Length Polymorphism digestion to identify alleles. Genomic DNA (4 µl) was amplified in a 25 µl PCR reaction mix containing Promega flexi green buffer 5 µl, dNTPs 0.5 µl, upstream and downstream oligonucleotide primers 0.5 µl each, magnesium chloride 1.5 µl, 12.88 µl of nuclease-free water and Taq DNA polymerase 0.06 µl. AT<sub>1</sub>R gene: Cycling conditions include an initial denaturation of 94°C for 2 min, followed by 40 cycles of a further denaturation at 94°C for 1 min, annealing at 60°C for 1 min, extension 72°C for 2 min, and a final extension of 72°C for 10 min.<sup>[3]</sup>

A1166C polymorphism of the angiotensin 11 type 1 receptor primer sequence.<sup>[3]</sup>

5'-AAT GCT TGT AGC CAA AGT CAC CT-3'

5'-GGC TTT GCT TTG TCT TGT TG-3'

A cocktail of 0.25 µl of the Dde1 enzyme, 1 µl of the 10 × buffer D; 0.1 µl of acetyl BSA and 8.5 µl of sterile water was added to 10 µl of the PCR product. The enzyme digestion was performed in a final volume of 19.85 µl at 37°C for 4 h.

### Statistical analysis

The statistical package for social sciences-SPSS for windows® version 16.0 was used to statistically analyze

the data obtained. Genotype frequencies in control and hypertensive groups were compared by Chi-square analysis. Continuous variables were compared between hypertensives and controls by independent *t*-test. Multiple regressions analysis were also carried out using Systolic Blood Pressure or Diastolic Blood Pressure as dependent variable, then sex, age, and BMI were used as independent variables.  $P < 0.05$  was considered statistically significant.

## Results

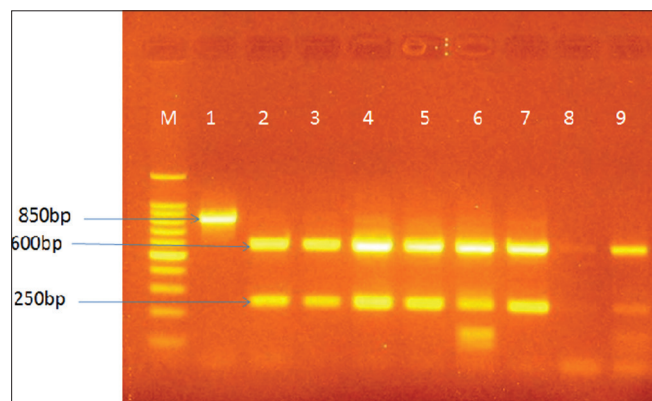
### PCR/enzyme digestion

**AT1R gene polymorphism:** The Dde1 enzyme cuts the PCR product into two pieces, 600 bp and 250 bp in the A variant. An additional Dde1 recognition site is created in the C variant at nucleotide 1166 located within the 250 bp fragment. The homozygous CC individual produces three bands (600 bp, 140 bp and 110 bp long). The homozygous AA individual produces two bands (600 bp and 250 bp long). The heterozygous individual produces four bands 600 bp, 250 bp, 140 bp

**Table 1: Genotype and allele frequency distribution among hypertensives and controls**

Groups	N	Genotype frequencies			Allele frequencies	
		AA	AC	CC	A	C
Hypertensives	612	605	7	0	1217	7
		0.99	0.01	0	0.99	0.01
Controls	612	606	6	0	1218	6
		0.99	0.01	0	0.99	0.01

AA: A1166C homozygous individuals, AC: A1166C heterozygous individuals, CC: A1166C homozygous individuals



**Figure 1: Agarose gel electrophoresis showing digestion of the 850 bp PCR product by the Dde1 restriction endonuclease. Legend Lane M is the 100 bp DNA ladder Lane 1 is the PCR product Lane 2 is the homozygous AA individual Lane 6 is the heterozygous AC individual**

and 110 bp long.<sup>[3]</sup> The homozygous CC individual was not observed Figure 1.

### Genotype and allele distribution

For the A1166C allele, 99% of the study population had the wild type AA genotype and 1% was AC heterozygous carriers of the mutation Table 1. The females (55.4%; 64.4%) with the AA genotypes were more than the males (43.6%; 36.4%) in control and patient population respectively Table 2. When continuous variables were compared between hypertensive and control groups, significant differences existed between the age, BMI, systolic and diastolic blood pressure of controls and patients. By multiple regression analysis, age was the predictor for SBP and DBP in the hypertensive group  $r = 0.604$  SBP,  $r = 0.594$  DBP,  $P < 0.05$ . Age was a predictor for DBP in the control group  $r = 0.542$ , gender was a predictor for SBP in the control group. The A1166C gene was not a positive predictor for hypertension in the study population.

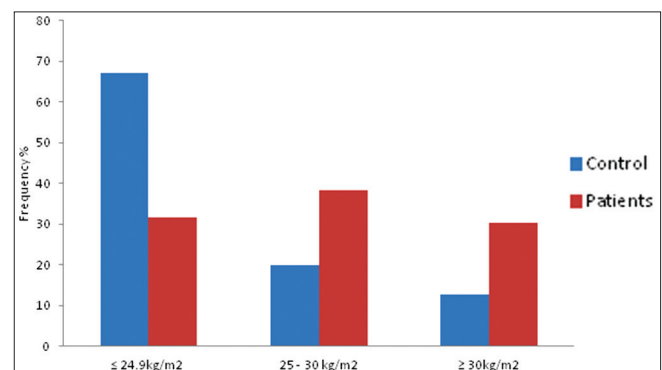
### Body mass index

In the patient population, BMI below 24.9 kg/m<sup>2</sup> was observed in 234 persons (31.54%), BMI between 25 kg/m<sup>2</sup> and 30 kg/m<sup>2</sup> was observed in 193 persons (38.23%) and BMI above 30 kg/m<sup>2</sup> was found in 185 persons (30.23%). In

**Table 2: Distribution of genotypes by gender among hypertensives and controls**

Genotypes	Control		Patients	
	Males %	Females %	Males %	Females %
AA	43.6	55.4	36.4	64.4
AC	0.7	0.3	0.2	0.8
CC	0	0	0	0

AA: A1166C individuals, AC: A1166C individuals, CC: C1166C individuals



**Figure 2: Body Mass Index observed among control and patient groups. Legend: Normal ≤ 24.9 kg/m<sup>2</sup> Overweight 25-30 kg/m<sup>2</sup> Obese ≥ 30 kg/m<sup>2</sup>**

the controls, BMI above 30 kg/m<sup>2</sup> was found in 80 persons (13.07%), BMI between 25 kg/m<sup>2</sup> and 30 kg/m<sup>2</sup> was found in 121 persons (19.77%) and a BMI below 24.9 kg/m<sup>2</sup> was observed in 411 persons (67.16%) Figure 2.

## Discussion

The CC allele of the AT1R gene was not observed in this study population. 99% of the population had the AA wild type gene. This is in contrast to what is reported in some literature. A high prevalence of the CC genotype was observed in Chinese hypertensives than controls.<sup>[16]</sup> In a sample of Swedish twins, Iliadou *et al.*,<sup>[17]</sup> did not observe any association between the ACE I/D polymorphism or AT1R A1166C polymorphism and blood pressure. Schmidt *et al.*,<sup>[18]</sup> also did not detect any association between the A1166C allele and hypertension but a decreased prevalence of C allele was observed among hypertensives. Tirt *et al.*<sup>[19]</sup> reported a higher prevalence of the C allele among female hypertensives than controls but no such observation among men. A higher prevalence of the CC genotype was observed in Chinese hypertensives than controls.<sup>[20]</sup>

Generally large interethnic differences in the frequencies of genotype polymorphisms of the RAS exist in different populations. To explain ethnic differences observed in allele frequency in different study populations, Lynch *et al.*<sup>[21]</sup> reported that there are differences in linkage disequilibrium for different loci among various populations if such differences exist in regions where these variants are found, and such variants are not causal but are in linkage disequilibrium with the putative locus, this might explain the inconsistency in results across population. Among the Efiks and the Ibibios, the major ethnic group in this study population, the frequency of the A allele was 99.7 and 0.3, for C allele the average C allele frequency reported in the Chinese population was 0.11. In another study, the homozygous A1166 allele frequency was 92.8 among the studied subjects. The frequency of homozygous A allele was significantly higher in the hypertensives than the normotensive subjects (97.5% and 80% respectively) with a higher frequency among male patients.<sup>[22]</sup>

Farrag *et al.*,<sup>[22]</sup> proposed that the A allele may be a predisposing factor for essential hypertension in Egyptians. Zhenni *et al.*,<sup>[23]</sup> observed only two genotypes AA and AC of the AT1R polymorphism, but reported a higher frequency of the A allele among the patients than the controls. Lee and Kim,<sup>[24]</sup> observed 96% and 6% for A and C allele of the angiotensin 11 type 1 receptor polymorphism in Korea. Hahntow *et al.*<sup>[25]</sup> reported the A allele to be associated with high blood pressure. He also reported that the A allele showing an association was not totally out of place given the fact that this locus had no major impact on hypertension phenotype as suggested by genome wide studies.<sup>[26-28]</sup> Other studies<sup>[2,17]</sup> have reported the C allele instead to be associated with hypertension.

Most patients were married and advanced in age (above 40 years). Increase in age is thought to increase blood pressure because the arteries become hardened, less active, kidney function decreases and the body does not process salt as well as before.<sup>[29]</sup> It was observed that most individuals below the age of 40 years were normotensives.

Most controls in the study had a BMI less than or equal to 24.9 kg/m<sup>2</sup> (67.10%). In the patient population, overweight 38.23% and obesity 30.23% reach a moderate prevalence among patients. Hypertension has been reported to be strongly correlated with BMI. Weight gain in adulthood is seen as an important risk factor for hypertension.<sup>[30]</sup> Humayun *et al.*,<sup>[31]</sup> observed a high trend of hypertension with increasing BMI among Pakistanis, with a high incidence among females whose weight was above normal that is more than 24.9 kg/m<sup>2</sup>. Positive associations between body mass index and blood pressure have also been documented in cross sectional studies in different Asian population. Ethnic differences existed in the association between BMI and hypertension and optimal mi cutoffs for overweight Chinese, Indonesians and Vietnamese adults.<sup>[32-34]</sup>

## Conclusion

The A1166C polymorphism is not associated with hypertension in the study population of Calabar and Uyo.



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