Original Article

Insertion/deletion polymorphism of the angiotensin-converting enzyme gene and the risk of hypertension among residents of two cities, South-South Nigeria

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Abstract

Background: Hypertension is a public health challenge due to its high prevalence, and is a major risk factor for cardiovascular diseases. This study was designed to determine the frequency of the I/D polymorphism of the angiotensin-converting enzyme gene and its association with hypertension in a sample population of Calabar and Uyo, South-South Nigeria.

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. The I/D polymorphism was investigated using polymerase chain reaction. Multiple regression and odds ratio (OR) was applied to test whether the ID genotypes were predictors of hypertension.

Results: The I/D genotype frequencies were 73(12%), 262(43%) and 277(45%); 74(12%), 303(50%) and 235(38%) for the II, ID, DD genotype in patient and control groups, respectively. A higher frequency of the ID genotype was observed in controls of which 208(61%) were females. By multiple regression analysis, age was a predictor for SBP in patients, $r = 0.596$, and DBP in controls, $r = 0.555$. Gender, Body mass index, I/D genotypes were not significant predictors for hypertension but the I/D polymorpism was associated with an increased risk for hypertension with an OR of 1.15 95%CI (0.924-1.456).

Conclusion: The I/D polymorphism of the angiotensin-converting enzyme gene was a risk factor for hypertension in the sample population of Calabar and Uyo. This research will form baseline information for subsequent molecular studies in this population.

Key Words: Angiotensin-converting enzyme gene, frequency, genotype, hypertension, I/D polymorphism

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INTRODUCTION

Angiotensin-converting enzyme (ACE) a key enzyme in the renin-angiotensin-aldosterone pathway is found in the kidneys. It catalyzes the conversion of angiotensin 1 to a physiologically active angiotensin 11 that controls fluid electrolyte balance and systemic blood pressure.[1] The ACE gene has been mapped to chromosome 17q23. The Insertion/Deletion (I/D) polymorphism was

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discovered in 1990 and was as a result of the presence of (insertion) or absence (deletion) of a 287-AluYa5 element inside intron 16 producing three genotypes: II homozygote, ID heterozygote, DD homozygote[2] Though the polymorphism is located in a non- coding region of the ACE gene, several investigators[3-7] have observed that the polymorphism is not silent but the DD homozygote is associated with increased activity of ACE in the serum and several diseases including hypertension.

The *ACE* gene was also implicated in the etiology of hypertension. The gene-coding area carries an ID polymorphism within intron 16. Several studies have associated the ACE I/D polymorphism with elevation in blood pressure in the Japanese and other ethnic groups.[8-10] Some studies have shown that this polymorphism is strongly associated with increased blood pressure in males;[11-13] however, a negative association was also detected in some linkage and association studies.[14,15] Gupta *et al*.[16] reported a negative association between the ACE polymorphism and hypertension in a rural population in India. The relationship between ACE and environmental factors predisposing to EH has been investigated in 1099 subjects from one Mongolian population. This study showed evidence for an interaction between the ACE DD (deletion/deletion) and ID polymorphism and cigarette smoking, alcohol drinking and BMI -body mass index.[17] No such studies have so far been reported for the residents of Calabar and Uyo, Nigeria. Hence, this study was carried out to identify the I/D polymorphism in the population and its possible association with hypertension.

MATERIALS AND METHODS

Calabar and Uyo are the capital cities of Cross River and Akwa Ibom states, respectively. These states are sister states that are part of the old Calabar Kingdom. The major ethnic groups in Calabar are Efik, Ejagham and Bekwara. In Uyo, the major groups are Ibibio, Annang, Oron, Ibeno and Eket. These different groups though distinct bear striking similarities in their culture and there is a lot of human migration between the two cities.

A population based association–case control–study was used to determine the frequency of ACE I/D allele and its relationship with hypertension status in Calabar and Uyo. A total sample population of 1224 adult men and women from different ethnic groups were 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 and the General Hospital, Calabar. The other 612 individuals served as the controls whose blood pressure was below 140/90 mmHg, who were not taking hypertensive drugs and not below the age of 20 from the same population.

Venous blood (3 ml) was collected from each participant and DNA was extracted from the blood for genotyping of the polymorphism. Subjects included in the study gave informed consent and ethical approval for the study was obtained from the joint UI/UCH ethical review committee and each of the health establishment concerned. All information obtained was treated as confidential. The wall in the collection center was calibrated in meters. Individuals stood without foot or head wear facing the investigator, looking straight ahead and the investigator placed a ruler on top of the head of the individual and the reading in meters was recorded. Readings was taken using a sphygmomanometer in millimeters of mercury by certified medical personnel for the patients in the clinics and a certified nurse for the controls in the general population. Systolic and diastolic B*P* values were recorded. Before taking the measurement, the respondent was advised to sit quietly for 5 min, with the legs uncrossed and the right hand free from clothing. The right hand was placed on the table with the palm facing upwards. The cuff was wrapped and fastened securely. The cuff was kept at the same level as the heart during measurement. The upper reading, the systolic blood pressure (SBP), and lower reading, the diastolic blood pressure (DBP) were recorded; the first and second readings were taken twice and the average of the two used for the analysis.

DNA was extracted as previously reported in Kooffreh *et al*.[18] The DNA was re-suspended in 50 µl of Tris-EDTA (T.E) buffer and stored in the freezer as a stock solution until further use. The ACE genotype was determined by amplifying genomic DNA in a polymerase chain reaction (PCR) using the primer pair 5'-CTG GAG AGC CAC TCC CAT CCT TTC T-3'; 3'-GAC GTC GCC ATC ACA TTC GTC AGA T-5'. Genomic DNA (2 µl) was amplified in a 12.5 µl reaction mix containing Promega flexi green buffer 2.5 µl, dNTPs 0.25 µl, upstream and downstream oligonucleotide primers 0.25 µl each, magnesium chloride 0.75 µl, 6.44 µl of nuclease-free water and Taq DNA polymerase 0.06 microliters. An initial denaturation for 5 min at 94°C was followed by 30 cycles of 45 sec at 94°C, 45 sec at 56°C and 45 sec at 72°C and a final elongation of 10 min at 72°C. 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. Multiple regression analysis was also carried out using SBP or DBP as dependent variable, then sex, age, BMI were used as independent variables. Odds ratio was calculated; $P > 0.05$ was considered statistically significant.

RESULTS

PCR was performed on the 612 control and 612 patient samples collected from Uyo and Calabar to determine the frequency of the I/D gene polymorphism and its relationship with hypertension status. Agarose gel allows the visualization of a 490 bp band for a homozygous individual with the insertion (I) allele and a 190 bp band for a homozygous individual with the deletion (D) allele. The heterozygous individual was identified by the presence of the 190 bp and the 490 bp PCR products [Figure 1].

Demographic data

There were a total of 1,224 subjects recruited into the study, consisting of 612 hypertensives—225 males and 387 females and 612 normotensives—272 males and 340 females [Table 1]. The Efiks and the Ibibios (34.2; 32.4% respectively, n = 612) were the main ethnic groups among the patients. The Ibibios (37.1%, n = 612) were the predominant ethnic group among the controls.

Genotype and allele frequencies

For the I/D allele of the ACE gene, the deletion was 45% and 38% (homozygous), the carriers of the deletion were 43% and 49% in the patient and control population, while the insertion allele was 12% in both control and patient populations. Among the Efiks which are the predominant ethnic group in Calabar town, the genotype frequency was 11%, 43%, 46% and 16%, 45%, 39% for the II, ID, DD genotypes among patients and controls, respectively. Among the Ibibios who also happen to be the predominant ethnic group in Uyo town, the genotype frequencies were 11%, 40%, 49% and 13%, 49%, 38% for II, ID, DD frequencies. The frequency of the D allele was 0.62 in the control subjects and 0.68 and 0.69 in the patients [Tables 2 and 3]. The observed genotype frequencies did not conform to the frequencies predicted by the Hardy-Weinberg theory. There were no significant differences between the genotype frequencies of hypertensive and the control

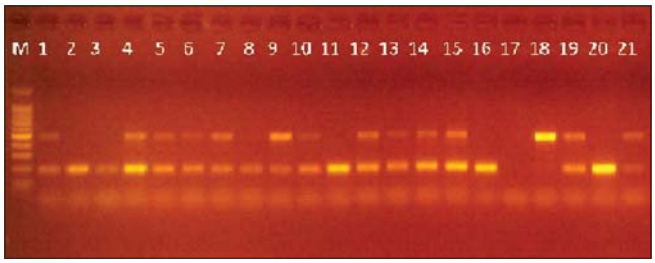

Figure 1: Agarose gel illustrating the amplification of the Insertion/Deletion of the angiotensin converting enzyme gene. Lane M is the 100bp DNA ladder. Lane 1,4,5,6,7,9,10,12,13,14,115,19,20 showed the 490bp and 190bp PCR products which were heterozygote individuals. Lane 2, 3, 8,11,16,21 showed the 190bp PCR products which were individuals with the homozygous deletion allele. Lane 17 showed no amplification and had to be repeated. Lane 18 showed the 490bp PCR product was an individual with the homozygous insertion allele

Table 1: The characteristics of the study population

	Patients	Controls
Gender		
Males	225	272
Females	387	340
Blood pressure		
Diastolic	93.25±13.77	72.18±8.41
Mean		
Systolic	161.14±13.26	116.76±9.19
Age		
Mean	51.3±13.76	31.9±10.27
Body mass index		
Mean	27.48±5.81	23.32±5.83

Odds ratio=1.15 (95% CL 0.924-1.456)

Table 2: Genotype and allele frequencies of the ACE polymorphism in the patient and control populations

	Total		ACE Polymorphism					
			Genotypes				Allele	
			II	ID	DD		I	D
Patients	612	No. of individuals	73	262	277			
		Genotype frequencies %	12	43	45	Allele frequency	0.33	0.67
Controls	612	No. of individuals	74	303	235			
		Genotype frequencies %	12	50	38	Allele frequency	0.37	0.63

ACE: Angiotensin-converting enzyme, II: Insertion/Insertion, ID: Insertion/deletion, DD: Deletion/Deletion

Table 3: Genotype and allele frequencies of the ACE polymorphism in the two major ethnic groups

Ethnic groups	Total			ACE polymorphism					
				Genotypes				Allele	
				II	ID	DD		I	D
Efik	Patients	209	No. of individuals	22	91	96			
			Genotype frequencies %	11	43	46	Allele frequency	0.32	0.68
	Controls	173	No. of individuals	27	78	68			
			Genotype frequencies %	16	45	39	Allele frequency	0.38	0.62
Ibibio	Patients	198	No. of individuals	22	80	96			
			Genotype frequencies %	11	40	49	Allele frequency	0.31	0.69
	Controls	227	No. of individuals	30	112	85			
			Genotype frequencies %	13	49	38	Allele frequency	0.38	0.62

ACE: Angiotensin-converting enzyme, II: Insertion/Insertion, ID: Insertion/deletion, DD: Deletion/Deletion

groups by χ^2 analysis. By multiple regression analysis, age was a predictor for SBP in patients r = 0.596 and DBP in controls r = 0.555. Gender, body mass index, I/D genotypes were not significant predictors for hypertension. The I/D polymorphism was associated with an increased risk for hypertension with an odds ratio (OR) of 1.15 95%CI (0.924-1.456).

In the control population, a higher frequency was observed for the ID genotype (51%) in females than their male counterparts (48%). In the patient population, the genotype frequencies were 12%, 43%, 45% in females and in males for the II, ID, DD genotype, respectively [Table 4].

Blood pressure

For patients the mean diastolic blood pressure was 93.25 ± 13.768, the mean systolic blood pressure was 161.14 ± 23.247. For the controls, the mean systolic blood pressure was 116.76 ± 9.19; the mean diastolic blood pressure was 72.181 ± 8.41.

DISCUSSION

The frequency of the I/D polymorphism of the ACE gene was determined and its association with increased risk for hypertension was investigated in a sample population in Calabar and Uyo cities. ACE genotype frequencies were 12%, 43% and 45% and 12%, 50%, 38% for the II, ID, DD respectively in the patient and the control groups respectively. A higher frequency of the ID allele was observed in controls of which 172 (51%) were females. Among the major ethnic groups residing in the two towns, the D allele frequency was between 62% and 69% while the I allele was between 31% and 38%. Rotimi *et al*.[19] reported the frequency of the D allele among African Americans as 63% while Morshed and Akhteruzzman[20] reported 69.3% for the D allele in hypertensives and 45.7% in controls. He also observed a higher frequency of the I allele in the controls (54.2%) than the hypertensives (50%). Wang *et al*.[21] reported the D allele frequency to be 40.8% which is lower than what was obtained by O'Donnell *et al*.[11] in European samples (55.3%). Kario *et al*.[22] reported a frequency of 34% for Japanese individuals. Dankova *et al*.[23] reported 0.53 frequency for the mutant D allele in Slovak subjects and 0.447 in Romany subjects. A frequency of 52.9% for patients and 56.3% for controls was reported for the D allele in a Lebanese diabetic cohort by Chmaisse *et al*.[24] Ismail *et al*.[25] reported a significantly higher frequency (0.55) of the ACE II genotype in the hypertensive group than in the control group of the same age but no overall significant differences were observed between the II, ID, DD ACE genotypes. The D allele has been associated with hypertension in some studies in White American and Japanese men but not in women.[11,26] Sagnella *et al*.[27] observed a significant association between the D allele and hypertension in women of African descent. Many studies have failed to establish an association between the D allele and hypertension.[14-16,28] However, a strong association of the I allele was found in an Australian population with familial hypertension.[29] The conflicting results of the I/D polymorphism of the ACE gene in hypertension has been attributed to gender and ethnic differences.[6]

Table 4: Genotype and allele frequencies among gender in the study population

Gender		Total		ACE Polymorphism					
				Genotypes				Allele	
				II	ID	DD		I	D
Males	Patients	225	No. of individuals	28	96	101			
			Genotype frequencies %	12	43	45	Allele frequency	0.34	0.66
	Controls	272	No. of individuals	36	131	105			
			Genotype frequencies %	13	48	39	Allele frequency	0.37	0.63
Females	Patients	387	No. of individuals	45	166	176			
			Genotype frequencies %	12	43	45	Allele frequency	0.33	0.67
	Controls	340	No. of individuals	38	172	130			
			Genotype frequencies %	11	51	38	Allele frequency	0.36	0.64

ACE: Angiotensin-converting enzyme, II: Insertion/Insertion, ID: Insertion/deletion, DD: Deletion/Deletion

In this study, the I/D allele of the ACE gene is associated with an increased risk for hypertension with an odds ratio of 1.15 (95% CI, 0.924 -1.456). Ji *et al*.[30] observed a higher odds ratio of 1.61 (95% CI, 1.32-1.98) for the ACE gene among the Han Chinese population. Sagnella *et al*.[27] reported an odds ratio of 1.65 (95% CI, 1.04-2.64) in women of African descent (OR = 2.54; 95%CI = 1.38-4.65) but not in men of African descent (OR = 0.79; 95% CI, 0.36-1.72). Bhavani *et al*.[31] reported a significant association of the ACE I/D allele with hypertension in men with age adjusted OR of 2.25 (95% CI, 1.14-4.42) and 2.20 (95% CI, 1.22-3.80) for DD and ID, respectively. In women there was no significant association of ACE genotype with hypertension, age adjusted odds ratio being 1.20 (95% CI, 0.38-3.92) and 0.44 (95% CI, 0.17-1.06) respectively for the DD and ID genotypes. Das *et al*.[10] observed that the odds of being hypertensive in a population of Asian Indians of Calcutta was 7.48 (95%CI, 1.75-30.190) in the DD homozygous individual suggesting a very strong association of

the ACE polymorphism with essential hypertension in Asian Indians. Samper *et al*.[7] observed a strong association between the ACE polymorphism and hypertension among the peoples of Kashmir, India.

World distribution of the D allele according to Salem[4] suggest that the I/D polymorphism in the human ACE gene is of African origin. The allele is believed to have moved out of Africa with Paleolithic (second part of the stone age that began about 750,000 to 500,000 BC and lasted until the end of the ice age about 8,500BC) migrations 100.000 years ago. The ACE I/D polymorphism is due to an insertion of a 287bp AluYa5 element into intron 16 of the gene.[2] This insertion is believed to have occurred a few million years ago during the evolution of primates.[32] Although an insertion or a deletion event is implied in the I/D polymorphism, only an insertion event occurred. This makes the D allele without an insertion the ancestral state of the gene. Primate specific Alu elements have been reported to be the most abundant transposable elements in the human genome making up more than 10%.[33] The mechanism by which D allele leads to blood pressure elevation is not clearly documented in literature.

The genotype frequencies observed in this population did not conform to the Hardy- Weinberg theory suggesting the action of evolutionary mechanisms on this gene locus. Future research would be to search for other genes that act in concert with the ACE gene to produce disease in the study population.

CONCLUSION

The Insertion/Deletion polymorphism of the angiotensin-converting enzyme (ACE) gene was associated with an increased risk for hypertension. Thus, the ACE gene polymorphism is a molecular marker for hypertension in the study population. This research will form baseline information for subsequent molecular studies in this population.

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