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Polymorphism of the atrial natriuretic peptide hormone and hypertension in residents of Calabar and Uyo, Nigeria.

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ABSTRACT

The natriuretic peptide systems also affect blood pressure through its vasodilatory and natriuretic activities generating interest in its role in the development of hypertension. The polymorphisms of the ANP have been investigated in different populations but similar studies are not documented for Nigerian populations. The study investigate the association of –C664G allele of the ANP gene and hypertension in residents of Calabar and Uyo, Nigeria. The study involved 1308 participant of which 612 were patients and 696 were controls from the population. Allele specific polymerase chain reaction and restriction enzyme digestion was used to genotype the population. The –C664G mutation was not observed in this population. This study adds to the data on the –C664G polymorphism and hypertension in human population.

INTRODUCTION

The heart plays an important role in regulating salt and water balance. This is mediated by a cardiac hormone referred to as the atrial natriuretic peptide (ANP) or factor (ANF), a potent natriuretic and vasorelaxant hormone that is mainly secreted by cardiomyocytes plays a role in cardiovascular homeostasis. When blood sodium and blood pressure levels increase, ANP secreted from the heart bind to its receptors in the kidney and blood vessels, promotes the excretion of salt, lowers blood volume and relaxes the vessels. The heart and kidney are thus involved in maintaining a fine balance of electrolytes and body fluid. The ANP is a 28 amino acid peptide in humans that assumes a hairpin structure by virtue of a cystein bridge that links residues 7 and 23. Several nucleotide polymorphisms have been identified in the ANP gene. One of them is the –C664G polymorphism located in the promoter region. Rubattu *et al*, 2006 reported that the –C664G polymorphism is responsible for the down regulation of ANP gene transcription; it is associated with left ventricular hypertrophy in Italians. The –C664G has been reported to be monomorphic among the Chinese and no other SNPs are in linkage disequilibrum with the –C664G polymorphism (Xue *et al*, 2008).

Rubattu *et al*, 2007 found that young Italian men heterozygous for the G allele had an increased risk for an early onset of the disease. When compared with homozygous G individuals, carriers of the-664G mutation also had an increased left ventricular mass index in a study among a highly homogenous population of Caucasian patients (Rubattu *et al*, 2006). The C664G polymorphism showed a borderline association with hypertension in Japanese subjects (Kato *et al*, 2000). Hu *et al*, 2007 genotype 1186 individuals from the Matsu area in Taiwan, 35 years and above, no difference was observed between the allele frequency of patients and controls. Kato *et al*, 2002 did not observe any association between this polymorphism and stroke. This study addressed the question whether the –C664G gene variant of the atrial natriuretic peptide gene is associated with hypertension in residents of calabar and Uyo since genetic diversity exist among different populations and the fact that an association in one population cannot be extrapolated to another population.

METHODS

The study was performed with randomly recruited participants (1308) from the hypertension clinic in the teaching hospital, Calabar , the teaching hospital, Uyo and individuals residing within the cities. 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 696 were individuals whose blood pressure was below 140/90mmHg, who were 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. Resting blood pressure was measured after participants had rested for 10 mins. Blood pressure was read two times using a mercury spygnomanometer, the mean value of this measurement was used. The weight and height of participants were measured according to standerd procedure, body mass index was determined. All participants were questioned about their smoking habits, alcohol consumption, activity physical, medical family history and use of hypertensive medications. Ethical approval was granted by the university of Calabar teaching hospital, Calabar and the university of Uyo teaching hospital, Uyo, participants gave informed consent before taking part in the study. 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. DNA extraction was carried out according to Bereczky *et al*, (2005). Pieces (1-2) of the filter paper about 5mm in diameter were cut using a sterile blade for each samples. These pieces were placed in an eppendorf tube, soaked in 65µl of T.E buffer. The tube was incubated at 50°C for 15 mins 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 15mins at 97°C to elute the DNA. The liquid condensing on the lid and the walls of the tube were removed by a short centrifugation (2-3 secs).The DNA extract that is the supernatant was kept at -20°C before use. Genomic DNA (2µl) was amplified in a 25µl PCR reaction mix containing Promega flexi green buffer 5µl, dNTPs 0.5µl, upstream and downstream oligonucleotide primers 0.5µl each, magnesium chloride 1.5µl, 12.88µl of nuclease-free water and Taq DNA polymerase 0.06µl.

Cycling conditions include an initial denaturation of 95°C for 3 mins, followed by 35 cycles of a further denaturation at 94°C for 20 secs, annealing at 60°C for 30 secs, extension 72°C for 30 secs, and a final extension of 72°C for 5 mins.

$C^{664}G$ Polymorphism of the Atrial natriuretic peptide gene primer sequence

5' – AAC AGC AAC GGA AGA AAT GA -3'

5' – ATC CAA CCC CCA AAT AGA AGT A-3' (Kato *et al*, 2000).

A cocktail of 0.25µl of the Rsa1 enzyme, 1µl of the 10 x buffer E; 0.1µl of acetyl BSA and 8.5µl of sterile water was added to 10µl of the PCR product. The enzyme digestion was performed in a final volume of 19.85µl at 37°C for 4 hours. The digested products were separated on 2% agarose gel stained with 10µl of ethidium bromide for 30 mins at 125V. The Statistical Package for Social Sciences – SPSS for windows® Version 16.0 was used to statistically analyze the data obtained. Descriptive statistics was used to analyze all variables studied which include bodymass index, smoking practices, alcohol consumption in the study population. Continuous variables were compared between hypertensives and controls by independent t test.

RESULTS

The aim of this study was to genotyping a sample population of 1308 individuals to determine the frequencies of the C664G allele of the ANP gene and associate the allele with hypertension status. Polymerase chain reaction and enzymatic digestion was performed on the 696 control and 612 patient samples collected from Uyo and Calabar to determine the frequency of the C664G variant and its relationship with hypertension status.

For the Rsa1 RFLP, the enzyme cuts the PCR product into two pieces (134bp and 23bp). The common allele (C664C) individual gives an undigested 157bp; the minor allele carrier (C664G) gives two fragments of 134bp and 23bp. Minor allele individual (G664G) gives a 134bp fragment. However agarose gel allows the visualization of a 157bp fragment for common allele individual (plate 1), a 134bp fragment for the minor allele individual, a 157bp and 134bp for the minor allele carrier individuals respectively (Kato *et al*, 2000).

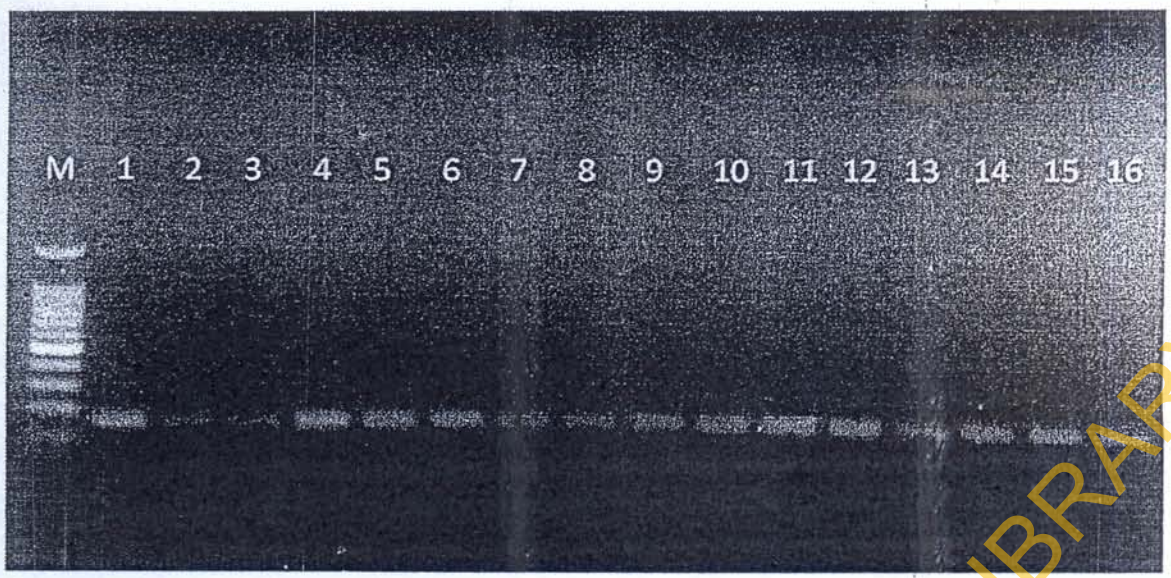

Plate 1 Agarose gel electrophoresis showing the undigested 157bp product after enzymatic digestion with RsaI restriction enzyme

The participants were made up of 612 hypertensives - 225 males and 387 females and 696 normotensives -273 males and 423 females, this is shown in fig 1. Table 1 shows a summary of the ethnic distribution of the patient and control groups. The Efiks and the Ibibios (34.2; 32.4% respectively, n=612) were the main ethnic groups among the patients. The Ibibios (41.5%, n=696) were the predominant ethnic group among the controls Fig 1.

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. According to the JNC classification on hypertension, 281 patients had stage one hypertension and 331 patients had stage two hypertension, for the systolic BP measurement. From the diastolic BP measurement, 381 patients were grouped into the stage 1 category and 231 patients had stage 2 hypertension. For the systolic BP measurement in controls, 395 were classified into the prehypertension group while 301 were classified as normal. For the diastolic BP measurement, 309 controls were classified into the prehypertension group and 387 controls as normal (table 2).

Among the cases group, 330 persons (53.9%) reported they do not have a positive history of hypertension.163 persons (26.6%) have a positive history of hypertension. 119 (19.4%) persons have no idea. None of the normotensives had any history of hypertension although 22.7% had a positive family history of hypertension. 26.6% reported a negative family history of hypertension, 50.7% had no idea if there was any history of familial hypertension.

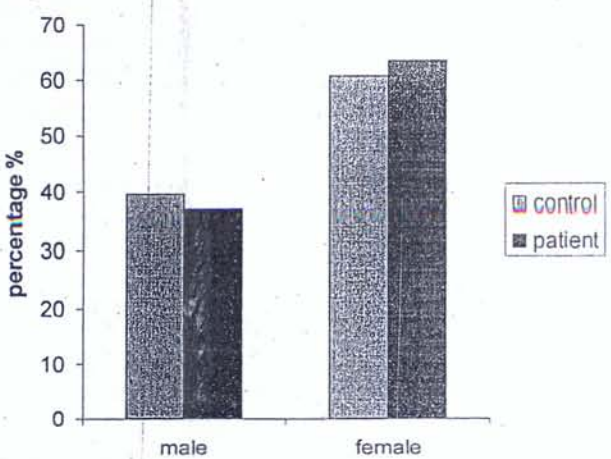

Fig. 1. Distribution by gender in the control and patient group

Table 1. Ethnic distribution of patient and control populations

Ethnic Groups	Patients		Controls	
	Frequency	Percentage%	Frequency	Percentage%
Efiks	209	34.2	175	25.1
Bekwara	15	2.5	21	3
Ejagham	19	3.1	23	3.3
Ibibio	198	32.4	289	41.5
Annang	49	8	121	17.4
Oron/okobo	24	3.9	17	2.4
Eket	7	1.1	0	0
Igbos	48	7.8	24	3.4
Others	14	2.3	8	1.1
Abi/Yakurr/Boki	24	3.9	17	2.4
Okoyong/Ekoi	5	0.8	1	0.1
	612		696	

Table 2. Classification of the patient and control population according to the JNC VII classification of blood pressure.

	no of individuals	groups according to JNC		
Patients:				
Systolic BP	281	stage one hypertension	Mean systolic BP	161.14±23.247
	331	stage two hypertension		
Diastolic BP	381	stage one hypertension	Mean diastolic BP	93.25 ±13.768
	231	stage two hypertension		
Controls:				
Systolic BP	395	prehypertension	Mean systolic BP	116.76±9.19
	301	normal		
Diastolic BP	309	prehypertension	Mean diastolic BP	72.181±8.41
	387	normal		

The patient ranged from 24 to 90 years old with a mean age of 51.4 years. Among the patient group, 464 (75.8) persons were more than 40 years of age and 148 (24.2) patients were less than 40 years.

Controls ranged from 20 to 73 years old with a mean age of 31.9 years, 569 (81.7%) controls were less than 40 years of age while 127 (18.3%) controls were above forty years. 603 (98.5%) of patients were non smokers, 678 (97.4%) of controls were non smokers. 434 (70.9%) of the cases do not consume alcohol, 19.1% consume very little alcohol occasionally. 394 (56.6%) of controls do not consume alcohol, 225 (32.3) take alcohol occasionally. 77 (11.1%) persons consumed alcohol on a regular basis

In the patient population, BMI below 24.9kg/m^2 was observed in 234 persons, BMI between 25 – 30kg/m^2 was observed in 193 persons and BMI above 30kg/m^2 was found in 185 persons. In the controls, BMI above 30kg/m^2 was found in 89 persons, BMI between 25 – 30kg/m^2 was found in 140 persons and a BMI below 24.9kg/m^2 was observed in 467 persons.

Only the C664G genotype was observed in the study population. When continuous variables were compared between patient and control groups, significant differences existed between the age, BMI, systolic and diastolic blood pressure of controls and patients.

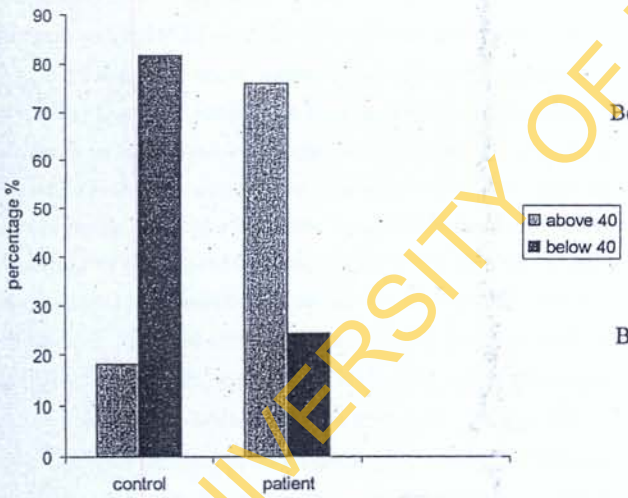

Fig 2 Age distribution of patient and control group

DISCUSSION

The aim of this study was to genotype some residents of Calabar and Uyo for the –C664G mutation that is associated with hypertension in some other populations. The -C664G mutant of the atrial natriuretic peptide gene was not observed in this population. The wild type allele of this polymorphism (-C664C) was present in both patient and control groups. This could imply that the mutation may not have occurred or had not been introduced into this population. This results have to be confirmed in a larger well defined population in the two cities. This observation is in line with a study among the Chinese the reported the ANP allele to be monomorphic (Xue *et al*, 2008) though it was associated with hypertension in some studies (Rubattu *et al*, 2006; Hu *et al*, 2007) Smoking and alcohol consumption was low in both populations. The patients had been educated by their doctors not to consume alcohol. The reason for abstinence among controls was due mainly to their religious beliefs. Most controls had a BMI less than or equal to 24.9 (467 persons, 67.10%), (140 persons, 20%) were overweight and (89 persons, 12.79%) were obese. In the patient population, 234 patients had normal BMI of ≥ 24.9, more patients were overweight (193, 38.23%) and obese (185, 30.23 %). Positive associations between body mass index and blood pressure have been well documented in both cross sectional studies in different populations (Stevens *et al*, 2008; Bell *et al*, 2002; Tuan *et al*, 2009).

CONCLUSION

The –C664G variant was observed in the study population and could not be associated with hypertension in the population

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