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APOLIPOPROTEIN GENE VARIANTS AND SUSCEPTIBILITY TO ESSENTIAL HYPERTENSION IN CAMEROON

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ABSTRACT

Background: **Essential hypertension (EH) is determined by both genetic and environmental factors and their complex interactions. Apolipoprotein (Apo) ε gene polymorphisms have been reported to be at the basis of EH in some ethnic groups but not in others. This inconsistency suggests that the association of Apo ε gene polymorphism could be dependent on the ethnic group. Cameroon is comprised of three ethnic groups and such studies have never been carried out in any of these groups despite a prevalence of 47.35% in the Cameroonian community.**

Objective: **To investigate the relationship between Apo ε gene polymorphisms and EH in the Bantu ethnic group of Cameroon.**

Design: **Cross sectional study.**

Setting: **Bantu ethnic group of South West Cameroon.**

Results: **Whereas advanced age, SBP, DBP, lack of exercise and family history constituted risk factors of EH, sex, body mass index (BMI), Fasting blood sugar (FBS), lipid profile, smoking, excessive alcohol consumption did not. Following conduction of association studies between Apo ε gene variants and risk factors of EH, statistical analyses of genotype and allele frequencies revealed that the frequency of ε4 allele was statistically higher in the hypertensive than the normotensive group.**

Conclusion: **The ε4 allele predisposes Cameroonians of the Bantu ethnic group to EH.**

INTRODUCTION

Hypertension is a complex multifactorial and polygenic disorder that is thought to result from an interaction between an individual's genetic makeup and various environmental risk (1, 2). Hypertension is a major risk factor for cardiovascular disease, stroke, and end-stage renal disease. Prevention of this disease is therefore an important public health goal. Essential hypertension (EH), also known as primary hypertension, is one of the most common complex diseases accounting for 95% of all cases of hypertension (3). One preventive approach is to identify disease-susceptibility genes that involve a specific physiological or cellular function, because elucidation of these genes will influence all efforts towards the management and prevention of EH (4).

There is a growing awareness that individuals with EH tend to have a high prevalence of associated dyslipidemias thereby linking lipoprotein metabolic abnormalities with EH. Polymorphism in the apolipoprotein (Apo) ε gene results in various isoforms that interact differently with specific lipoprotein receptors, ultimately altering the levels of cholesterol in the circulation (5). The Apoε gene is coded for by three common alleles;ε2, ε3 and ε4 (6, 7). Each individual inherits one allele from each parent resulting to a homozygote: ε2/ε2 or ε3/ε3 or ε4/ε4 or heterozygote: ε2/ε3 or ε2/ε4 or ε3/ε4. Each of these three isoforms is defined by two single nucleotide polymorphisms (SNPs) at positions 2059 (T/C) and 2197 (C/T) of the Apo εgene (8). Thus ε2, ε3 and ε4 alleles have the SNP genotype: TT, TC, CC respectively and when digested with the HhaI restriction enzyme will give a characteristic banding pattern. From these alleles, arise six genotypes; ε2/ε2, ε2ε3, ε2/ε4, ε3/ε3, ε3/ε4 and ε4/ε4. (9). The ε3/ ε3 genotype is the most common and it is considered the wild- type Apo ε genotype because it has not been found to be associated with any pathological condition (10). While there are rare variants, it is the polymorphism of Apo ε gene with its three common alleles (ε2, ε3 and ε4)

that has been studied in relation to EH. There have been several studies of the association between the Apo ε genotypes and prevalent EH, with inconsistent findings.

Results from small prevalent case control studies have consistently described a positive relationship between the presence of the ε4 allele and hypertension or with greater blood pressure (BP) levels (3, 11). The results from cross-sectional studies have been more inconsistent. Four investigations carried out in a mixed population of younger and older adults reported: a lack of association between the Apo ε genotype and EH in the USA (12). and in Tunisia (13), a positive association between the ε2 allele and prevalent EH among males, but not females, Japanese immigrants living in Los Angeles or Hawaii (14), and a negative association between the presence of the ε4 allele and prevalent hypertension in young, but not older, Japanese (15). Three other cross-sectional studies included very old subjects. Two were carried out in Finland (16) and in Spain (17), and did not find significant association between Apo ε genotype, prevalent EH (17), or mean SBP and mean DBP (16). The third study included Italian descendants living in the south of Brazil and reported a significantly lower DBP among the ε4 carriers, compared to the homozygous ε3/ε3, but the mean SBP was similar in both groups (18). In addition, two cohort studies carried out with Japanese Americans (14) and South-eastern Brazilians (19) reported contradictory results.

Identification of markers of EH in ethnic settings like Cameroon which is made up of three main ethnic groups; the Bantu (South West, Centre, South, East and Littoral Regions), Semi-Bantu (North West and West Region) and Foulbe (Far North, North and Adamawa Regions) (20), may reveal specific Apo ε variants that are markers of EH in each ethnic group. Hence, this study seeks to elucidate the relationship that could exist between the Apo ε gene polymorphism with EH in the Bantu ethnic group in South West of Cameroon.

MATERIALS AND METHODS

Study design: This was a cross sectional population-based study with participants randomly selected on a home-to-home basis. Ethical clearance was obtained from the Cameroon Bioethics Initiative of Ethics Review and Consultancy Committee (CAMBIN- ERCC) in Yaounde and administrative authorization was obtained from the South West Regional Delegation of Public Health. Informed consent was obtained form all participants.

Study population: Participants comprised of 119 adults aged 35- 65 years, of the Bantu population of SWR of Cameroon and were randomly recruited on a home-to-home basis for this study. All participants were unrelated, of purely Bantu descent for at least 3 generations and whose ancestors had lived in the SWR for at least three generations.

Participants were distributed into two groups: normotensive and hypertensive subjects. Hypertensive subjects (n=67) were defined as those having a systolic blood pressure (SBP) of ≥140 mmHg and a diastolic blood pressure (DBP) of ≥90 mmHg, a family history of hypertension and are currently on anti-hypertensive medication.

Healthy control subjects (n= 52) of the same region with a SBP of less than 140 mmHg and a DBP of less than 90 mmHg but without any history of EH, diabetes and other immunosuppressive conditions were enrolled as normotensives.

All individuals below the age of 35 or above 65 years, developed any form of complications as a result of hypertension as well as those who developed diabetes before the onset of hypertension were excluded from the study.

Collection of demographic, anthropometric, biological and biochemical data: A certified nurse used structured questionnaires for collection of both demographic (age, sex, family history, alcohol consumption) and anthropometric data (height & weight) (21). The body mass index (BMI) was calculated according to Quetelet equation (22). For acquisition of biological data two morning systolic (SBP) and diastolic (DBP) blood pressure measurements were taken using a sphygmomanometer (Omron health care, Illinois, USA) (23). A third measurement was taken only when the difference between the two measurements was greater than 5 mmHg, and the readings were averaged for analysis. A 5-min. relaxation period between measurements was maintained for all subjects.

For biochemical data, fasting blood sugar (FBS) was determined in mg/dl glucose using OneTouch UltraMini stripTM and analyzer (Carlifornia, USA). Serum triglycerides (TGs) were determined by enzymatic colorimetric method using a commercially kit (ChronoLab, Barcelona, Spain) according to the manufacturer's instructions. Total cholesterol (TC) and high density lipoprotein cholesterol (HDL-C) were determined using a Kit from ChronoLab (Barcelona, Spain) according to the manufacturer's instructions. Serum LDL was calculated using the formula: LDL-C = (TC) - (HDL-C) - (TG/5) (24).

Apo □ gene polymorphism: Two milliliters of whole blood were collected by venepuncture from patients in EDTA-microtainer tubes and preserved at 4oC for less than 12 hours until DNA extraction. Genomic DNA was extracted manually from human whole blood by non-enzymatic salting out method (25). Genomic DNA was re-suspended in 0.5 mL of 0.1 M TE buffer and stored at at -20oC until used for polymerase chain reaction (PCR). PCR amplification of Apo □ gene was carried out in a 2720 Thermal

cycler (Applied Biosystems, Singapore) using a pair of gene specific primers flanking the Apo ε gene to yield 218bp (19, 3). PCR was performed in 100 µL PCR tubes with 1 µL of 10µM each primer (Apo □Fwd: 5′-TCCAAGGAGCTGCAGGCGGCGCA-3′, Apo □ Rev: 5′-ACAGAA TTCCGCCCCGGCCTGGTACACTGCCA-3′) in a final volume of 25 µL containing 0.5 µL genomic DNA suspensions (0.2µg), 12.5 µL of PCR master mix and 10 µL of distilled water. PCR master mix (Sigma) contained other components of PCR (1x Taq buffer, 0.05 U/µL Taq polymerase, 2.0 mmol/L MgCl2, 0.4 mmol/L deoxynucleotides triphosphates and 10 x DNA loading dye). PCR was done with an initial denaturation at 94oC for 5 min, then the DNA was amplified for 30 cycles with denaturation at 94oC for 45 sec, annealing at 70oC for 45 sec. and extension at 72oC for 45 sec. followed by a final extension at 72oC for 5 min. The amplified DNA (15 µL) was digested for 5 hours at 37oC with 10U Hha I restriction enzyme (New England Biolabs). Restricted fragments were analysed on 8% nondenaturing polyacrylamide gel and stained with silver (26).

Statistical analysis: Data were analysed with Centre for Disease Control (CDC) epidemiological software (EPI INFO) version 7. All tests were two-side and a $p< 0.05$ was considered statistically significant at a 95% confidence interval. Significance differences between group means were tested by Student t-test and differences in proportions was assessed by Chi-square test. Allelic frequencies were calculated by gene-counting method and the genotype distribution with Hardy-Weinberg expectations by a Chi-Square test. Odds ratios with 95% confidence intervals (CI) were estimated for the effects of high-risk alleles. Univariate analysis was used to determine independent association of genotype or allele frequency with EH.

RESULTS

Anthropometric, biological and biochemical data assessed to be potential risk factors of EH are summarized in Table 1.

Table 1
Anthropometric, biological and biochemical characteristics of normotensive and hypertensive subjects

Parameter	Cases (N= 67)	Controls (N= 52)	P- value
Gender: male/female	31/36	28/24	0.41219
Age: Years	52.69±12.02	46.41±10.73	0.004*
SBP (≤140mmHg)	151.07±17.12	97.38±9.21	0.000*
DBP (≤90mmHg)	118.10±11.90	79.02±7.62	0.000*
BMI (≤ 30Kg/m^2)	29.14±5.35	28.20±5.10	0.40120
RBS (≤200mg/dl)	149.08±49.505	138.73±34.14	0.470
TC (≤ 200mg/dl)	211.95±97.07	216.74±99.12	0.798
TG	127.20±116.84	109.45±77.22	0.356
(Men= 60-160mg/dl)			
(Women= 35-135mg/dl)			
HDL-C (≤ 65mg/dl)	68.13±25.16	68.80±23.44	0.886
LDL-C (≤150mg/dl)	116.64±96.81	118.54±98.86	0.924
Family history (%)	31 (54.39)	16 (31.37)	0.012*
Feeding habit (%)	10 (27.78)	17 (37.78)	0.343
Smoking (%)	7 (12.96)	8 (15.38)	0.721
Alcohol consumption (%)	43 (82.68)	47 (90.38)	0.250
Lack of exercise (%)	28 (52.83)	38 (73.08)	0.030*

Risk factors found to be statistically significant were age, SBP, DBP, family history and lack of exercise. These parameters were significantly different between the hypertensive than normotensive subjects. For all other risk factors such as sex, FBS, TC, TG, HDL-C, LDL-C, feeding habits, smoking and alcohol consumption, differences were not statistically significant between the two study groups (Table 1).

Genotype and allele frequencies of Apo ε gene polymorphism: Genotyping of the Apo ε gene by restriction fragment length polymorphism resulted into six different genotypes: ε3/ε3, ε2/ε3, ε3/ε4, ε4/ε4, ε2/ε4, ε2/ε2 (Figure I) in diverse proportions (Table 2)

Figure 1
Gel-separated products of apoE HhaI digestion. Each genotype possesses unique combinations of HhaI fragment sizes. The ε2/ε2 (91 and 83 bp); ε3/ε3 (91 bp, as well as 48 and 35 bp fragments; ε4/ε4 (48 and 35 bp) well as a unique 72 bp fragment. (The 19 bp fragment was too small for detection and are not represented on the gel).

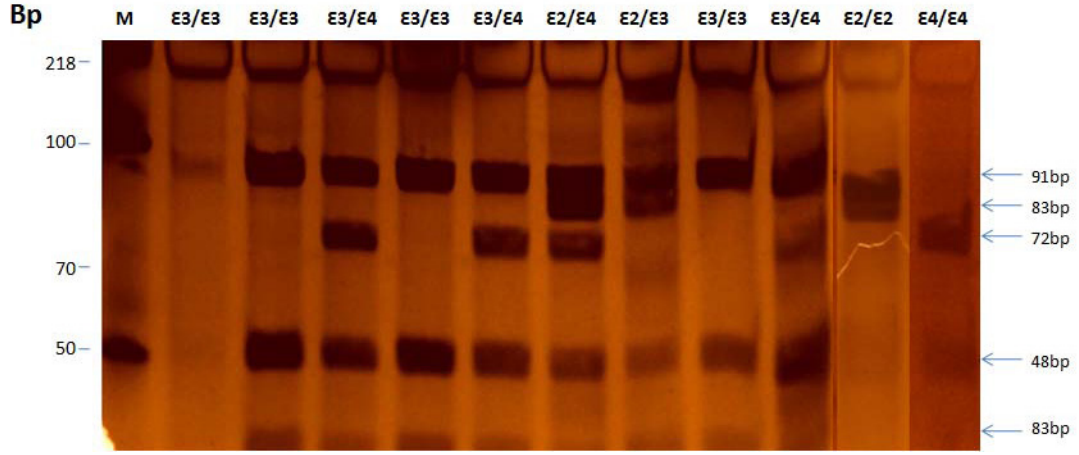

Table 2
SNPs and genotypes of each allele with HhaI-digested fragments

Allele	SNPs	SNP Genotype	Digested fragments (Bp)
ε_2	2059 (T/T), 2197 (C/T)	TT	91, 83 and 35
ε^2	2059 (T/T), 2197 (C/C)	TC	91, 48 and 35
ε^3	2059 (T/C), 2197 (C/C)	CC	72, 48 and 35
ε^4			
$\varepsilon_2/\varepsilon_3$	2059 (T/T), 2197 (C/T)	TT	91, 83, 48 and 35
	2059 (T/T), 2197 (C/C	TC	
$\varepsilon_2/\varepsilon_4$	2059 (T/T), 2197 (C/T)	TT	91, 83, 72, 48 and 35
	2059 (T/C), 2197 (C/C)	CC	
$\varepsilon_3/\varepsilon_4$	2059 (T/T), 2197 (C/C	TC	91, 72, 48 and 35
	2059 (T/C), 2197 (C/C)	CC	

Table 3
Genotype and allele distributions of Apo-E in hypertensive and normotensive groups

		Genotype						Allele		
		$\varepsilon_3/\varepsilon_3$	$\varepsilon_2/\varepsilon_3$	$\varepsilon_3/\varepsilon_4$	$\varepsilon_4/\varepsilon_4$	$\varepsilon_2/\varepsilon_4$	$\varepsilon_2/\varepsilon_2$	ε_3	ε_4	ε_2
Hyp (N=67)	**n**	11	11	**29**	4	11	1	62	**48**	24
	%	16.42	16.42	43.28	5.97	16.42	1.49	46.27	35.85	17.91
Nor (N=52)	**n**	24	6	**11**	2	8	1	65	**23**	16
	%	46.15	11.54	21.15	3.85	15.38	1.92	62.5	22.11	15.38
P		0.34	0.62	0.01*	0.53	0.72	0.88	0.25	0.02	0.24

Hyp: Hypetensive subjects & Nor: Normotensive subjects

Of the 67 hypertensive subjects, 16.42 % had the homozygous ε3/ε3 genotype, 43.28 % had the ε3/ε4 heterozygous genotype, 16.42 % had the heterozygous ε2/ε3 genotype, 5.97 % had the homozygous ε4/ ε4, 16.42 % had the ε2/ε4 heterozygous genotype and 1.49 % was homozygous for the ε2/ε2 genotype (Table 3). Also, of the 52 normotensive subjects, 46.15 % had the homozygous ε3/ε3 genotype, 21.15 % had the ε3/ε4 heterozygous genotype, 11.54 % had the heterozygous ε2/ε3 genotype, 3.85 % had the homozygous ε4/ε4 genotype, 15.38 % had the ε2/ε4 heterozygous genotype and 1.92 % was homozygous for the ε2/ε2 genotype. For allele frequency, 46.27 % of hypertensive subjects had the ε3 allele, 35.28 % had the ε4 allele and 17.91 % had the ε2 allele while 62.5 % of the normotensive subject had the ε3 allele, 22.11 % had the ε4 allele and 15.38 % the ε2 allele. (Table 3).

The genotype distributions for the polymorphisms were in H–W equilibrium (P> 0.05). Chi-squared test revealed significant statistical differences in genotype and allele frequency (P < 0.05) between normotensive and hypertensive subjects with genotype ε3/ε4 and allele ε4 standing out as risk factors of EH (with a 95% CI, p < 0.05) in the study population (Table 3).

DISCUSSION

The inconsistency in previous findings on the association of Apo ε gene polymorphism and EH is thought to result from ethnic and geographical variations (27, 28). The present study was aimed at investigating the association of Apo ε polymorphism with EH among the Bantu ethnic population in the SWR of Cameroon. The identification of this polymorphism in this subset of the Cameroonian population as a health risk factor will help people predisposed to EH to take adequate health decisions in order to prevent the disease and its complications. A subpopulation analysis according to potential risk factors of EH revealed that age SBP, DBP, lack of exercise and family history were risk factors in the Bantu ethnic group while gender, BMI, lipid profile, alcoholism and smoking were not (Tables 1). EH was more prevalent in the elderly than young people. This was in accordance with previous studies in different towns of Cameroon (29, 30). Increase in age is thought to increase blood pressure because as the arteries become hardened there is increased resistance to blood flow (31).

The high SBP and DBP in hypertensive patients were naturally expected. These results reveal that SBP and DBP are risk factors for EH in the Bantu ethnic population of SWR Cameroon. In recent years, considerable evidence has suggested that changes in vascular endothelial function may cause the increase in vascular tone, in addition to sympathetic activity and increased circulating levels of angiotensin II (32). The proportion of individuals that did not make exercise or with a family history of EH was statistically different between the hypertensive patients and control population, suggesting that genetic and lifestyle environmental factors do influence the ability to develop this disease in this ethnic group. These observations are in line with earlier reports (11), thereby providing evidence that heritable factors in combination with a number of recognized environmental risk factors are important determinants of the pathogenesis of EH in this study population.

The PCR products from both populations were successfully restricted with HhaI restriction enzyme and from the restriction pattern, six genotypes were detected: ε3/ε3, ε3/ε4, ε2/ε3, ε4/ε4, ε2/ε4, ε2/ε2, with their corresponding molecular weight varying from 34 to 91bp (Figure 1). The ε3, ε2 and ε4 allele frequencies were calculated from genotype frequencies. The ε4 allele was significantly prevalent in the hypertensive group as compared to their normotensive counterparts (p=0.02 at a 95% CI). This was not the case for ε3 allele (p=0.25). Similar results were obtained for the ε3/ε4 genotype. From the results of this study, it was evident that the allele ε4 and the genotype ε3/ε4 could predispose individuals of this population to EH considering a p=0.01 between the two groups . Considering that the allele ε3 difference was not found to be significant between the two groups, the effect in the ε3/ε4 genotype could come from the ε4 allele. This study therefore suggests that the ε4 allele could predispose individuals to EH.

These results are similar to those obtained in the Chinese (3), Indian (11) and Italian populations living in the South of Brazil (18), where there was a significant difference in genotype and allele distribution between hypertensive and control subjects. On the other hand, this observation was not in line with those obtained in Brazil where there was no significant association between Apo ε genotypes and prevalent EH amongst older males (19), in USA there was a lack of association between the Apo ε genotype and EH in a mixed population of younger and older adults (14) and in Tunisia there was also a lack of association between Apo-E genotype and HTN (13). These discrepancies confirm the fact that predisposition of EH due to polymorphism of the Apo ε gene depends on in ethnicity and geographical location.

In summary, this study revealed a strong correlation between Apo ε allele ε4 and predisposition of individuals of the Bantu ethnic group of Cameroon to EH. It will be necessary to investigate the relationship between the Apo ε allele and EH in different ethnic groups as well as the role this allele plays in the molecular mechanism of pathogenesis of EH in these populations.

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