The atrial natriuretic peptide gene and essential hypertension in African-Caribbeans from St Vincent and the Grenadines

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Atrial natriuretic peptide (ANP) which alters sodium balance, blood volume and vascular tone represents an important candidate for investigating the genetic basis of essential hypertension (EH). Accordingly, we have studied *Bgl*I and *Xho*I restriction fragment length polymorphisms (RFLPs) of the ANP gene in 147 hypertensive, 141 normotensive and 67 population-based control

subjects from a homogenous population of West African origin from St Vincent and the Grenadines. We found no association of either *Bgl*I and *Xho*I RFLPs with EH. This study suggests that the ANP locus may not exert a major gene effect on EH amongst the black people of St Vincent and the Grenadines.

Keywords: essential hypertension; homogenous black population; atrial natriuretic peptide; polymorphism

Introduction

Studies of populations, families and twins suggest that the aetiology of essential hypertension (EH) may have a significant genetic contribution.^{1–5} The continuous distribution of blood pressure (BP) in several populations implies that EH may be polygenic and does not follow simple mendelian modes of inheritance.^{6–8} However, the number and kind of genes involved in the pathogenesis of EH remain unknown.

EH appears to be associated with an inherited difficulty in salt excretion.⁹ This difficulty seems to be comparatively marked in people of African ancestry who also appear to be more sensitive to the BP raising effects of salt.¹⁰ The genetic defects in salt regulating systems which play a role in the aetiology of EH may therefore cluster in certain African populations.

Atrial natriuretic peptide (ANP) may influence BP regulation through enhancing urinary sodium and water excretion, relaxation of vascular smooth muscle, increased transfer of fluid into the extracellular compartment and antagonism of actions of the renin-angiotensin-aldosterone system (RAAS) and noradrenaline.^{11–13} Animal models of hypertension suggest the possibility that in salt-sensitive persons, certain variants of the ANP receptor and perhaps ANP itself may predispose to EH.^{14,15} The ANP gene

therefore represents an attractive candidate for investigation into the genetic determinants of EH.

A previous study in a heterogeneous urban European black population found no association between the ANP locus and EH.¹⁶ Accordingly, we sought to determine whether there was any association between polymorphisms at the ANP locus and EH in a homogenous black population from St Vincent and the Grenadines.

Subjects and methods

Volunteers

Following ethical approval, 147 hypertensive and 141 normotensive subjects were identified through the Kingstown General Hospital and General Practices of St Vincent and the Grenadines. All the subjects were adults with both parents and all grandparents of African origin. BP measurements were done according to the British Hypertension Society recommendations.¹⁷ The hypertensive subjects had begun to have high BP before the age of 60 years and diastolic BP (DBP) above 95 mm Hg on two occasions, or were on antihypertensive therapy with documented DBP greater than 95 mm Hg. Hypertensive subjects also had a positive family history of EH. Documented parental or sibling hypertension developing before the age of 60 years was regarded as positive family history. Those with secondary hypertension as defined by clinical evidence of intrinsic renal disease or renovascular hypertension and diabetes were excluded. Subjects with creatinine clearance (enzymatic) above 1.34 mL/s/m² (for

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males) and 1.20 mL/s/m² (for females) were also excluded. The normotensive subjects had DBPs below 85 mm Hg on two occasions and a negative family history of hypertension and diabetes. Normotensive subjects with a documented history of parental or sibling hypertension were excluded. A randomly selected population-based control group of 67 black Vincentians was identified to provide baseline data. All subjects were interviewed, their weights and heights measured and a 30 mL venous blood sample was collected into tubes containing potassium EDTA from each one: 10 mL of this was centrifuged and the plasma transferred into fresh tubes for creatinine estimation. The rest of the sample was transported frozen and stored at -70°C until DNA was isolated and analyzed.

Laboratory

DNA purification and Southern analysis were carried out according to published procedures.¹⁸ A purified and radiolabelled DNA insert corresponding to a 1.0 kb human ANP genomic *Bam*H1/*Eco*R1 fragment subcloned in pBR327 plasmid was used for hybridization.¹⁸ Creatinine was determined commercially.

Statistical analysis

Anthropometric data of the hypertensive, normotensive and population-based control groups were expressed as mean $\pm$ standard deviation and compared using the unpaired Student's *t*-test. For each biallelic RFLP, genotype frequencies and their distributions between the hypertensive and normotensive subjects were compared using the χ^2 test to determine association with EH. Deviation of the study population from Hardy-Weinberg equilibrium was assessed by χ^2 test. Probability values of ≤ 0.0500 were regarded as indicating statistical significance.

Results

Clinical data

The anthropometric data of the hypertensive and normotensive control groups are summarized on Table 1. The hypertensive subjects were signifi-

cantly ($P < 0.0001$) older and had significantly ($P < 0.0001$) greater body mass index (BMI) than the normotensive subjects. The hypertensive men and women were older and had greater BMI compared with the normotensive men and women. Within the hypertensive group, the females though younger ($P = 0.7351$), had significantly greater ($P = 0.0473$) BMI. This was also the case in the normotensive subjects. In the hypertensives, 88% of the subjects were 45 years old or above; 67% of the normotensives were 45 years or above. The population-based control group had a mean age (42.9 ± 15.86) which was below the mean ages of both the hypertensive and normotensive subjects. The males and females in the population-based control group had no statistically significant differences in their ages ($P = 0.1124$), systolic BPs (SBP) ($P = 0.1860$), DBPs ($P = 1184$) and body mass indices ($P = 0.5938$).

Genetic data

The following RFLP allele sizes were detected; 12 kb (allele 1) and 10 kb (allele 2) with *Bgl*I DNA digestions, and 6.2 kb (allele 1) and 4.1 kb (allele 2) with *Xho*I digestions. For both *Bgl*I and *Xho*I alleles, the genotype frequencies were comparable to those of a population in Hardy-Weinberg equilibrium.

***Bgl*I polymorphism:** In hypertensive and normotensive groups as well as in the population-based control group, the smaller 10 kb (*B*₂) allele was the more frequent and the proportion of the alleles in the three groups were similar. The results of the ANP *Bgl*I polymorphism are summarized on Table 2. Three *Bgl*I genotypes were obtained from the two alleles. The rare allele homozygous genotype (*B*₁*B*₁) was the least frequent while the heterozygous genotype (*B*₁*B*₂) was the most frequent in all three groups. The distribution of the *Bgl*I genotypes between the hypertensive and normotensive groups showed no statistically significant difference ($P = 0.3228$). However, the power of the study in this case to detect a significant association was only 23.88% which was below the desired power $\geq 80.00\%$. Genotype frequencies in the population-based control group were also similar to those

Table 1 Anthropometric characteristics of hypertensive and normotensive subjects

	<i>n</i>	Hypertensives				<i>n</i>	Normotensives			
		Age (years)	BMI (kg/m ²)	SBP (mm Hg)	DBP (mm Hg)		Age (years)	BMI (kg/m ²)	SBP (mm Hg)	DBP (mm Hg)
Age <45 years										
Women	10	40.1 $\pm$ 3.45	35.2 $\pm$ 6.41	165.0 $\pm$ 10.8	107.0 $\pm$ 8.23	25	37.2 $\pm$ 3.50	29.2 $\pm$ 7.00	121.6 $\pm$ 7.4	74.0 $\pm$ 6.12
Men	8	39.4 $\pm$ 2.67	30.4 $\pm$ 8.49	175.0 $\pm$ 30.71	106.9 $\pm$ 12.23	22	37.6 $\pm$ 3.16	24.6 $\pm$ 3.24	129.2 $\pm$ 8.09	76.4 $\pm$ 6.01
Age >45 years										
Women	66	63.4 $\pm$ 10.72	28.0 $\pm$ 6.62	174.2 $\pm$ 20.71	106.9 $\pm$ 9.61	46	57.4 $\pm$ 8.22	25.6 $\pm$ 8.22	125.5 $\pm$ 11.94	75.4 $\pm$ 6.45
Men	63	63.8 $\pm$ 10.74	26.5 $\pm$ 4.38	168.4 $\pm$ 16.68	104.2 $\pm$ 7.82	48	62.1 $\pm$ 11.34	23.3 $\pm$ 3.84	125.4 $\pm$ 16.63	70.9 $\pm$ 7.15
Total	147	60.7 $\pm$ 12.8	27.9 $\pm$ 6.21	171.1 $\pm$ 19.3	105.7 $\pm$ 8.96	141	52.3 $\pm$ 13.6	25.3 $\pm$ 5.11	125.3 $\pm$ 13.7	73.8 $\pm$ 6.86

Data are expressed as mean $\pm$ s.d.; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 2 Genotype frequencies in all hypertensives and normotensive subjects below and above 45 years

Genotypes		Hypertensives			Normotensives		
		Total (<i>n</i> = 147)	Age <45 years (<i>n</i> = 18)	Age >45 years (<i>n</i> = 129)	Total (<i>n</i> = 141)	Age <45 years (<i>n</i> = 47)	Age >45 years (<i>n</i> = 94)
<i>Bgl1</i> ^{a,b}	1 1	21 (14)	5 (28)	16 (12)	31 (22)	31 (66)	0 (0)
	1 2	82 (56)	10 (56)	72 (56)	69 (49)	16 (34)	53 (56)
	1 2	44 (30)	3 (16)	41 (32)	41 (29)	0 (0)	41 (44)
<i>Xho1</i> ^{a,b}	1 1	15 (10)	2 (11)	13 (10)	27 (19)	27 (57)	0 (0)
	1 2	73 (50)	8 (44)	65 (50)	66 (47)	20 (43)	46 (49)
	2 2	59 (40)	8 (44)	51 (40)	48 (34)	0 (0)	48 (51)

Genotype frequencies are indicated in absolute numbers (percentages). 1 and 2 (genotype column) represent the rare and frequent rare alleles, respectively.

n represents the number of subjects.

^a indicates that there is a statistically significant difference in the genotype frequencies in hypertensives below 45 years vs normotensives below 45 years of age, ($P < 0.05$).

^b indicates that there is a statistically significant difference in the genotype frequencies in hypertensives above 45 years of age, ($P < 0.05$).

observed in the hypertensive and normotensive groups (data not shown).

***Xho1* polymorphism:** Two *Xho1* alleles (X_1 and X_2) were also observed. The smaller 4.1 kb (X_2) was also the most frequent allele in all the three groups of subjects, and the proportions of both alleles in the hypertensive, normotensive and the population based control group were similar. Distributions of the *Xho1* genotypes (X_1X_1 ; X_1X_2 ; X_2X_2) obtained from the two alleles were similar in the hypertensives and normotensives (Table 2) as well as in the population-based control group (population-based control group data not shown). There was no statistically significant difference in the distribution of genotypes between hypertensive and normotensive groups ($P = 0.1852$), although the power of the study in this case to detect significant association was only 34.29% which was also below the desired power of 80.00%.

In the normotensives, there was a complete absence of both *Bgl1* and *Xho1* homozygous frequent allele genotypes (B_2B_2 and X_2X_2) in subjects below 45 years and of the homozygous rare allele genotypes (B_1B_1 and X_1X_1) in those 45 years old and above (Table 2).

When subjects were stratified according to their ages, the distribution of *Bgl1* and *Xho1* genotypes between hypertensives and normotensives 45 years or above, was statistically significant (*Bgl1*, $P = 0.0010$; *Xho1*, $P = 0.0034$). Similarly, the distribution of *Bgl1* and *Xho1* genotypes for both hypertensives and normotensives below 45 years was also significantly different (*Bgl1*, $P < 0.0001$; *Xho1*, $P < 0.0001$) (Table 2). The χ^2 test was sufficiently powerful to detect significant differences in the proportions of both *Bgl1* and *Xho1* genotypes when the hypertensive and normotensive groups were stratified according to age.

Classification of the hypertensives according to the severity of EH shown in Table 3 revealed no statistically significant differences between hypertensives with DBPs above or below 100 mm Hg (*Bgl1*, $P = 0.0589$; *Xho1*, $P = 0.2142$).

Table 3 Genotype frequencies and severity of disease among hypertensive subjects

Genotype		DBP up to 100 mm Hg (<i>n</i> = 85)	DBP above 100 mm Hg (<i>n</i> = 62)
<i>Bgl1</i>	1 1	12 (14%)	9 (15%)
	1 2	53 (62%)	29 (47%)
	2 2	20 (24%)	24 (39%)
<i>Xho1</i>	1 1	7 (8%)	8 (13%)
	1 2	40 (47%)	33 (53%)
	2 2	38 (45%)	21 (34%)

No statistically significant differences (*Bgl1*, $P = 0.0589$; *Xho1*, $P = 0.2142$) in the genotype distributions below and above DBP (diastolic blood pressure) of 100 mm Hg.

Discussion

This study has ascertained the distributions of *Bgl1* and *Xho1* ANP RFLPs in the Vincentian population studied. Considering the distributions of similar alleles in another black population in a previous report,¹⁶ it would appear that the proportions of these alleles are similar within black populations regardless of their geographical location, but conserved in proportions which are different in white populations.^{16,19}

The allele and genotype frequencies in the general population as represented by the randomly selected population-based control group, did not differ significantly from the frequencies observed in either the hypertensive or normotensive subjects, suggesting that in this population, possession of either the frequent or rare alleles does not predispose to EH. This is consistent with reports from the few previous studies of the ANP locus.^{16,19} Despite the role of ANP in salt metabolism, BP control, and its specific antagonism of many pressor activities,^{11–13} the *Bgl1* and *Xho1* variants of the ANP gene do not appear to generate major defects in BP. This probably indicates that ANP does not produce its actions

at a level where malfunction can have the long term effects on BP control which is associated with EH.

The ability of this exploratory investigation to affirm the ANP locus as a disease susceptibility locus for a complex trait like EH depends on its contribution to the variability of BP in the Vincentian population. This is not known. However, this investigation was carried out with the assumption that the ANP locus was a major locus in the aetiology of EH with zero recombination between marker and disease locus. With these assumptions, the power of this investigation to detect significant association between ANP locus and EH was only about 34%. This investigation does not therefore rule ANP out as a disease susceptibility locus in EH.

Even where it is possible to have a sufficiently large number of subjects, several other factors may affect the results of genetic investigations into a complex trait like EH. Mismatching the hypertensive and normotensive groups regarding age and BMI may be partly responsible for the negative results obtained.²⁰ Being an age related disease, the older section of the population produced most of the volunteers. Under the retrospective conditions this investigation was conducted, it is not possible to perfectly match the large number of hypertensive and normotensive subjects. The choice of the markers may have also caused the lack of association between the ANP locus and BP and the complete absence of the homozygous genotypes in the normotensives classified by age.

The lack of genetic association between EH and the ANP locus in this and other studies, together with the fact that some positive associations have been reported between certain pressor loci and EH^{21,22}, may decrease interest in the role of counterpressor systems in the pathogenesis of EH. However, this and the few previous studies were not designed to indicate whether the ANP locus can modulate biological response to salt intake. Different results may therefore be obtained when subjects are classified according to their response to dietary salt. A transgenic animal model with disrupted proANP gene has shown that the ANP gene modulates BP responses to dietary salt.¹⁵ This indicates that other variants of ANP not yet studied in humans may be useful in the identification of hypertensive patients likely to benefit from reduced salt intake. Furthermore, the significant differences found in this study when subjects were stratified according to their ages may be important, and suggest that careful stratification of subjects and the use of well defined intermediate phenotypes may substantially increase the power of population studies using smaller numbers of volunteers.

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