Journal of Human Hypertension (2001) 15, 425–430

www.nature.com/jhh

ORIGINAL ARTICLE

T594M and G442V polymorphisms of the sodium channel β subunit and hypertension in a black population

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Polymorphisms of the epithelial sodium channel may raise blood pressure by increasing renal sodium reabsorption. This study examines frequency distributions and associations with hypertension of the T594M and of the G442V polymorphisms of the β subunit of the epithelial sodium channel in a population-based sample. We studied a stratified random sample of 459 subjects (279 women), aged 40–59 years, of black African origin from general practices' lists within a defined area of South London. All were first generation immigrants. The polymorphic variants were detected using single strand conformational polymorphism technique (SSCP). The prevalence of hypertension (BP ⩾160 and/or 95 mm Hg or on drug therapy) was 43%; of these, 76% were on drug therapy. The main analysis was carried out by three ordered blood pressure categories (I to III) according to increasing blood pressure and presence or absence of drug therapy. The frequency of the 594M variant (heterozygotes and homozygotes) was 4.6%; the frequency of the 442V variant was higher (27.0%). The frequency of the 594M variant increased with increasing blood pressure category ($P = 0.05$) and was more common in hypertensives than normotensives. By contrast the frequency of the 442V variant did not vary across increasing blood pressure categories ($P = 0.62$). No gender difference was observed. Adjustment for age, sex and body mass index did not alter these findings. These results suggest that the 594M variant may contribute to high blood pressure in black people of African origin whereas the G442V polymorphism is unlikely to influence blood pressure in this population.

Journal of Human Hypertension (2001) **15,** 425–430

Keywords: genetics; epidemiology; black people of African origin; sodium channel

Introduction

Hypertension is common in black populations of African descent living in urbanised environments.[1] It is a particular problem in Africans and Caribbeans living in the United Kingdom[1,2] with stroke and end-stage renal failure being the major causes of death and disability.[3–6] In a recent population-based survey of cardiovascular risk factors in ethnic minority groups we found that nearly 50% of middle-aged black people (both West Africans and Afro-Caribbeans) had high blood pressure requiring treatment.[2–7]

High blood pressure in blacks is associated with low plasma renin activity, an index of sodium retention and volume expansion, and appears to be more sensitive to changes in sodium intake.[8] Previous studies have shown that black people have a slower sodium excretion in response to intravenous sodium infusion suggesting the possibility of a defect in the control of renal sodium excretion. Moreover, there is now evidence of a strong heritable component of salt-sensitivity in blacks.[9] These observations therefore suggest the possibility of a molecular defect in renal sodium handling.

Similarities in some phenotypic expressions between hypertension in blacks and patients with Liddle's syndrome,[10] a monogenic form of hypertension, have suggested that abnormalities of the distal tubular epithelial sodium channel—a major regulator of the overall control of sodium balance—may underlie the development of high blood pressure in blacks.[11] A number of variants of the sodium channel β subunit coding sequences have been identified in subjects with hypertension.[12–14] These lead to a single amino acid change rather than a major truncation as seen in Liddle's mutations and are much more frequent in blacks than in whites, especially the most commonly identified T594M and G442V polymorphisms.[12]

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Received 13 July 2000; revised and accepted 27 November 2000

In a previous case-control study we found that the 594M variant of the last exon (exon 12) of the β subunit of the epithelial sodium channel was more frequent in hypertensive than normotensive blacks.[15] In this study we also found that black people with the 594M variant had suppressed plasma renin activity when compared to wild-type individuals suggesting a state of corrected volume expansion.[15] It is not known, however, whether other sodium channel variants are also associated with high blood pressure in blacks and whether the presence of two or more sodium channel variants have a greater effect on sodium transport and blood pressure.

The G442V polymorphism, a mutation of exon 8 of the sodium channel β subunit, is of particular interest as it was previously identified in 10 out of 50 black hypertensives of African origin and in only one out of 475 hypertensive whites.[12,16] The specific objectives of the present study, therefore, were to extend our previous work on the T594M and to investigate the association with blood pressure across a range of increasing blood pressure levels of both the T594M and the G442V polymorphisms of the β subunit of the epithelial sodium channel in a large population-based sample of middle-aged black people of African origin living in London.

Subjects and methods

Population

The population sample was obtained as previously described.[2,7] In brief, the Wandsworth Heart & Stroke Study[2,7] is a population-based cross-sectional survey of 1577 men and women aged 40–59 years obtained from age and sex-registers of general practitioners in a defined area of South London. Fieldwork was undertaken from March 1994 to July 1996. Ethnic group was recorded at the time of the interview, based on the answers to a combination of questions including country of birth, language, religion, history of migration and parental country of birth.[7] All black participants of African origin (both West Africans and Afro-Caribbeans) were first generation immigrants. The study protocol was approved by the local Ethics Committee. All participants gave their informed consent to participate. The T594M polymorphism was determined in 458 (83.4%) and that of the G442V polymorphism in 459 (83.6%) out of the 549 (279 women) black people who took part in this study. The baseline characteristics of those not genotyped were comparable to those who were (data not shown).

Protocol

Participants attended a dedicated screening unit at St George's Hospital between 08.00 and 12.00, after an overnight fast. They were asked to refrain from smoking and from taking vigorous exercise for at least 1 h before the visit and to bring all drugs with them for checking. Anthropometry and blood pressure were taken with standardised methods as described elsewhere.[7] Fasting venous blood was taken in the seated position without stasis for serum biochemistry and plasma aldosterone measurement.[7] A questionnaire was administered which included personal medical history and drug treatment. After the interview, a complete 24-h urine collection was obtained as previously described[7] in 323 out of the 459 participants (70.4%). As the number of participants on antihypertensive medication was high (n = 149), the main analysis was carried out using three ordered blood pressure (BP) categories: I (BP $<$140 mm Hg and $<$90 mm Hg and not on drug therapy); II (BP $\geqslant$140 and $<$160 mm Hg or $\geqslant$90 and $<$95 mm Hg and not on drug therapy); III (BP $\geqslant$160 mm Hg and/or $\geqslant$95 mm Hg or being on drug therapy). Further analyses using measured blood pressure values were carried out in people not on regular drug therapy for hypertension.

Genetic analysis

Genomic DNA was isolated from whole blood as previously described using Nucleon BACC DNA extraction kit.[17] The 594M and 442V variants were detected using polymerase chain reaction (PCR) and single strand conformational polymorphism (SSCP) analysis. PCR and SSCP conditions for detection of the 594M variant were as previously established.[15,18] For this variant, a subset of the individuals in Group I (80%) was included in our previous clinic-based comparison,[15] however, for the present study, these were re-genotyped as part of the whole population-based sample. For detection of the 442V variant, 100 ng of genomic DNA was amplified using a sense primer (5′-CTCTTGGCCGCCTTTCTG-3′) and an antisense primer (5′-ATGCCTGCCCGCTGCTGTGC-3′) in a total volume of 25 μl reaction mixture containing 15 pmol of each primer, 200 μM dNTPs, 1.5 mM $MgCl_2$ and 0.25 U Redhot DNA polymerase (Advanced Biotechnologies). After an initial denaturation at 94°C for 5 min, amplification was carried out by 35 cycles at 94°C (1 min), 62.5°C (1 min) and 72°C (30 s), and a final extension at 72°C for 5 min. For detection of the variants by SSCP, 4 μl aliquots of each PCR product were denatured by addition of 8 μl denaturing solution (94% formamide, 10 mM NaOH, 0.25% bromophenol blue) and heating to 94°C for 5 min, followed by rapid cooling on ice. Samples were separated at 4°C by electrophoresis at 250V for 10 h on 0.8 $\times$ MDE (Flowgen) gels prepared in 0.6 $\times$ TBE and run in 0.6 $\times$ TBE running buffer. The DNA was visualised by silver staining. The ability of the SSCP method to detect the G442V polymorphism was confirmed by direct sequencing during the development and validation of this SSCP method. For this, PCR products were purified with spin columns (QIAGEN) and subjected to direct sequence analysis of both strands employing a dye terminator kit on an ABI 377 automated sequencer.

To prevent observer bias, those who carried out the genotyping (YBD and HDZ) were unaware of sample origin and all gels were cross-checked by a separate individual (GAS).

Statistical methods

Allele frequencies were tested for Hardy–Weinberg equilibrium using chi-square tests to compare observed against expected frequencies with df=1. Odds ratios (OR) and exact 95% confidence intervals (95% CI) were calculated using Epi-Info. Chi-square tests for trend in proportions with 1 degree of freedom were used to assess the relationship between genotype and blood pressure category.[19] Further analyses allowing for confounders were carried out using logistic regression with the SPSS-PC 8.0, in which hypertensives (category III) were compared with normotensives (categories I+II).

Results

Demographic and other characteristics of the whole population sample by gender, adjusted for age are given in Table 1. Men (n = 180) were slightly older than women (n = 279) (52.0 ± 5.6 [mean ± s.d.] *vs* 50.5 ± 5.8 years; P = 0.006). The prevalence of hypertension (defined as category III) was 43% (196/459) and was comparable in men and women (P = 0.98). The frequency of the 594M variant (heterozygotes plus homozygotes) was 4.6%. The genotype (TT, TM, MM) frequencies were 95.4%, 4.4% and 0.2% with allele frequencies of 97.6% and 2.4%, respectively. The 594M variant was found in 21 individuals, only one of whom was homozygous. The frequency of the variant increased with increasing blood pressure category (chi-square for trend, P = 0.05) and was more common in the hypertensive group (Table 2).

Higher frequencies of the M allele amongst hypertensives was also seen in men and women analysed separately, with OR of 3.93 (95% CI 0.69–29.1) and 1.68 (0.49–5.82)), respectively when comparing hypertensives (category III) with the other two groups (I+II) combined. Amongst Caribbeans (n = 289) the frequency of the 594M variant was 5.1% in normotensives (I + II) *vs* 6.1% in hypertensives (III). Amongst West Africans (n = 170) the frequencies were 0% *vs* 6.7% for normotensives and hypertensives, respectively. However, due to the small numbers, this sub-group analysis has limited statistical power. The analysis of the 11 untreated individuals out of the 21 possessing the 594M variant did not show any significant difference in weight, body mass index, serum electrolytes, creatinine and plasma aldosterone compared to those untreated without the 594M variant (Table 3). Twenty-four hour urinary sodium excretion also did not differ between hypertensives (n = 145) and normotensives (n = 178) (168 ± 68 *vs* 167 ± 65 mmol/24 h, mean ± s.d., P = 0.88) or between those with (n = 18) and without (n = 305) the 594M variant (177 ± 74 *vs* 166 ± 66 mmol/24 h, P = 0.52).

Table 1 Characteristics of men and women of black African origin

Parameters	*Men (n = 180)*	*Women (n = 279)*	*P*
Weight (kg)	79.4 (0.9)	76.3 (0.8)	0.012
Body mass index (kg/m^2)	26.6 (0.3)	29.5 (0.3)	<0.001
Blood pressure (mm Hg)			
Systolic	134.7 (1.4)	135.0 (1.1)	0.88
Diastolic	88.3 (0.7)	85.0 (0.6)	0.001
Serum[a]			
Sodium (mmol/l)	139.6 (0.2)	140.1 (0.2)	0.045
Potassium (mmol/l)	4.15 (0.02)	4.14 (0.02)	0.85
Creatinine (μmol/l)	103.8 (1.1)	84.4 (0.9)	<0.001
Plasma aldosterone[b] (pmol/l)	351 (14)	339 (11)	0.51

Results are expressed as age-adjusted means (s.e.). [a]n = 177 and n = 276 for men and women, respectively. [b]n = 169 and n = 270 for men and women, respectively.

The frequency of the G442V polymorphism was much higher than that of the T594M. The genotype (GG, GV, VV) frequencies were 72.9%, 24.8% and 2.2% with allele frequencies of 85.4% and 14.6%, respectively. However, in contrast with the frequency of the 594M mutation, the frequency of the 442V mutation was comparable in each blood pressure category (Table 2). Amongst those not on treatment, there were no significant differences in anthropometry, blood pressure and biochemistry according to G442V polymorphism (Table 4).

Adjustment for age, sex and body mass index did not alter the pattern of the associations with hypertension of either the T594M or the G442V polymorphims (data not shown).

Both variants were in Hardy–Weinberg equilibrium and were not in linkage disequilibrium (counts for genotype combinations were: TT/GG = 314; TT/GV = 112; TT/VV = 10; TM/GG = 18; TM/GV = 2; TM/VV = 0; MM/GG = 1; MM/GV = 0; MM/VV = 0). To examine the possibility of an interaction between these two variants, the presence of hypertension was analysed by the possible genotype combinations. Because of the small numbers in some subgroups only three genotype groups were identified for analysis according to prevalence of hypertension (category III). In these, hypertension was present in 40.1% (126/314) in those with TT/GG genotype, 45.9% (56/122) in those with the 442V variant (TT/GV+TT/VV) and 68.4% (13/19) in those with the 594M variant (TM/GG+MM/GG) (chi-square = 6.49, P = 0.039). These results are consistent with hypertension being more common in those with the 594M variant but do not suggest any significant interaction between these two variants.

Discussion

In the present study we examined the association between two polymorphisms (T594M and G442V) in

Table 2 Association between the T594M and the G442V polymorphisms and blood pressure categories

	Blood pressure categories[a]		
	I	*II*	*III*
T594M (n = 458)			
TT	191	65	181
TM+MM	5 (2.5%)	3 (4.4%)	13 (6.7%)
OR (95% CI)	1.00	1.76 (0.27, 9.33)	2.74 (0.89, 10.0)[b]
G442V (n = 459)			
GG	147	49	139
GV+VV	51 (25.7%)	19 (27.9%)	54 (28.0%)
OR (95% CI)	1.00	1.12 (0.57, 2.16)	1.12 (0.70, 1.80)[c]

[a]I (BP < 140 mm Hg and <90 mm Hg and not on drug therapy); II (BP ⩾140 and <160 mm Hg or ⩾90 and <95 mm Hg and not on drug therapy); III (BP ⩾160 mm Hg and/or ⩾95 mm Hg or being on drug therapy). [b]Chi-square for trend = 3.83, df = 1; P = 0.05). [c]Chi-square for trend = 0.24, df = 1; P = 0.62.

Table 3 Characteristics by T594M genotype for untreated participants

	TT (n = 297)	*TM (n = 11)*
Weight (kg)	76.4 (74.9, 77.8)	80.5 (73.0, 88.0)
BMI (kg/m^2)	27.8 (27.3, 28.3)	28.2 (25.7, 30.7)
Blood pressure (mm Hg)		
Systolic	130.1 (128.1, 132.2)	132.0 (121.4, 142.6)
Diastolic	84.3 (83.2, 85.5)	85.4 (79.4, 91.4)
Serum sodium[b] (mmol/l)	139.8 (139.5, 140.1)	139.9 (138.3, 141.5)
Serum potassium[b] (mmol/l)	4.17 (4.14, 4.20)	4.31 (4.15, 4.46)
Serum creatinine[b] (μmol/l)	91.0 (89.5, 92.4)	91.6 (84.1, 99.1)
Plasma aldosterone[c] (pmol/l)[a]	273 (257, 290)	241 (176, 328)

Results are expressed as age- and sex-adjusted means (95% CI). [a]geometric mean. [b]n = 293 and [c]n = 285 in TT group.

Table 4 Characteristics by G442V genotype for untreated participants

	GG (n = 228)	*GV+VV (n = 82)*
Weight (kg)	76.0 (74.4, 77.7)	77.8 (75.1, 80.6)
BMI (kg/m^2)	27.7 (27.1, 28.2)	28.3 (27.4, 29.2)
Blood pressure (mm Hg)		
Systolic	130.3 (128.0, 132.6)	129.7 (125.8, 133.6)
Diastolic	84.2 (82.9, 85.5)	84.7 (82.5, 86.9)
Serum sodium[b] (mmol/l)	139.8 (139.4, 140.1)	140.0 (139.4, 140.6)
Serum potassium[b] (mmol/l)	4.18 (4.14, 4.21)	4.19 (4.13, 4.24)
Serum creatinine[b] (μmol/l)	91.0 (89.3, 92.6)	91.0 (88.3, 93.8)
Plasma aldosterone[c] (pmol/l)[a]	276 (258, 296)	258 (230, 290)

Results are expressed as age- and sex-adjusted means (95% CI). [a]geometric mean. [b]n = 224, [c]n = 219 in GG group and n = 79 in GV+VV group.

the β subunit of the epithelial sodium channel and high blood pressure in black people of African origin. Our study has several important aspects in addressing genetic variations within a population. The sample has been taken within the same geographical area thereby mitigating the potential effects of differences in environmental background. It examines first generation immigrants with both parents born in the country of origin and belonging to the same ethnic background, thus reducing the potential impact arising from an unknown degree of admixture. Moreover, the design overcomes many of the problems of selection bias and differential misclassification which are potential causes of false positive results in clinic-based case-control studies of candidate genes.

The present work makes two important contributions: it provides population-based frequency estimates of the T594M and the G442V polymorphisms according to increasing blood pressure level and it also examines whether the presence of both the 442V and the 594M variants have a greater effect on blood pressure then when either is present alone. The G442V variant affects exon 8 of the sodium

channel β subunit and it results in a single amino acid change with substitution of valine (GTC) for glycine (GGC) at amino acid 442. Exon 8 encodes a segment of the extracellular loop and changes in this region would not be predicted to increase sodium channel activity. Indeed, while the 442V variant was more common than the 594M variant amongst black people, overall there were no significant differences in the frequency of the 442V variant according to blood pressure grouping. Moreover, there did not appear to be any positive interaction in relation to high blood pressure between the 594M and the 442V variants, although the relatively small numbers and the absence of some haplotypes, clearly limits the interpretation of the results regarding the presence of any interaction between these variants.

The observation of a significant trend of increasing prevalence of the 594M variant across increasing blood pressure categories is consistent with our previous case-control study of hypertensives compared to normotensives and suggests that its presence may contribute to raise blood pressure across the range. Two studies in black people have not shown an association between the T594M polymorphism and hypertension. In a study of African Americans, the overall frequency of the variant was comparable to our own estimate but it was not more common amongst hypertensives.[20] Furthermore, a linkage analysis of 63 affected sibling pairs in the Caribbean failed to show a linkage of the epithelial sodium channel genes to hypertension.[21] The reasons for these differences are still not clear but they may be explained by the greater degree of genetic admixture of African Americans compared to first generation immigrants to the UK and by the lack of statistical power in the linkage study.

The 594M variant affects the last exon of the C-terminal of the β subunit of the epithelial sodium channel and results in a single amino acid change with substitution of methionine (ATG) for threonine (ACG) at amino acid 594. The threonine residue at position 594 in the β subunit is a potential consensus target site for phosphorylation by protein kinase C (PKC) which downregulates sodium channels. Therefore the 594M variant could increase sodium channel activity by causing affected channels to become insensitive to negative regulation by PKC.[22]

In this study there were no significant differences in plasma electrolytes and aldosterone according to T594M genotype although these comparisons may have limited statistical power due to the relatively small sample size. However, plasma aldosterone is not a sensitive marker of volume status and the effect on sodium balance remains a possibility. In fact, there is some evidence that the 594M variant may influence sodium balance *in vivo* as in our previous study[15] plasma renin activity was significantly lower in hypertensive black people with the 594M variant than in hypertensive blacks without this variant. This suggests that individuals with the 594M variant may have a greater degree of volume expansion than other black hypertensive people without this variant but further studies are required to elucidate the mechanisms whereby such susceptibility is expressed and its potential therapeutic implications.

Acknowledgements

The Wandsworth Heart & Stroke Study has received support from the Wandsworth Health Authority, SW Thames Regional Health Authority, NHS R&D Directorate, British Heart Foundation, British Diabetic Association and The Stroke Association. Studies of the genetics of the epithelial sodium channel are supported by the British Heart Foundation. We thank MJ Rothwell for technical assistance for the DNA extraction. GAS, GAM, NDC, DGC and FPC are members of the St George's Cardiovascular Research Group. The study was presented at the Ninth European Meeting on Hypertension, Milan, 1999.

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