Association of hypertension with T594M mutation in β subunit of epithelial sodium channels in black people resident in London

E H Baker, Y B Dong, G A Sagnella, M Rothwell, A K Onipinla, N D Markandu, F P Cappuccio, D G Cook, A Persu, P Corvol, X Jeunemaitre, N D Carter, G A MacGregor

Summary

Background Liddle's syndrome is a rare inherited form of hypertension in which mutations of the epithelial sodium channel result in increased renal sodium reabsorption. Essential hypertension in black patients also shows clinical features of sodium retention so we screened black people for the T594M mutation, the most commonly identified sodium-channel mutation.

Methods In a case-control study, 206 hypertensive (mean age 48·0 [SD 11·8] years, men:women 80:126) and 142 normotensive (48·7 [7·4] years; 61:81) black people who lived in London, UK, were screened for T594M. Part of the last exon of the epithelial sodium-channel β subunit from genomic DNA was amplified by PCR. The T594M variant was detected by single-strand conformational polymorphism analysis of PCR products and confirmed by DNA sequencing.

Findings 17 (8·3%) of 206 hypertensive participants compared with three (2·1%) of 142 normotensive participants possessed the T594M variant (odds ratio [OR]=4·17 [95% CI 1·12–18·25], p=0·029). A high proportion of participants with the T594M variant were women (15 of 17 hypertensive participants and all three normotensive participants), whereas women comprised a lower proportion of the individuals screened (61·2% hypertensive, 57·7% normotensive). However, the association between the T594M variant and hypertension persisted after adjustment for sex and body-mass index (Mantel-Haenszel OR=5·52 [1·40–30·61], p=0·012). Plasma renin activity was significantly lower in 13 hypertensive participants with the T594M variant (median=0·19 ng mL^{-1} h^{-1}) than in 39 untreated hypertensive individuals without the variant (median=0·45 ng mL^{-1} h^{-1}, p=0·009).

Interpretation Among black London people the T594M sodium-channel β subunit mutation occurs more frequently in people with hypertension than those without. The T594M variant may increase sodium-channel activity and could raise blood pressure in affected people by increasing renal tubular sodium reabsorption. These findings suggest that the T594M mutation could be the most common secondary cause of essential hypertension in black people identified to date.

Lancet 1998; **351:** 1388–92

Blood Pressure Unit (E H Baker MRCP, Y B Dong PhD, G A Sagnella PhD, M Rothwell BSc, A K Onipinla MSc, N D Markandu RGN, F P Cappuccio MRCP, Prof G A MacGregor FRCP), **Department of Pharmacology** (E H Baker), **Department of Public Health Sciences** (D G Cook PhD), **and Medical Genetics Unit** (Y B Dong PhD, Prof N D Carter PhD), **St George's Hospital Medical School, London, UK; and Inserm U36, College de France, Paris, France** (Prof X Jeunemaitre PhD, A Persu MD, Prof P Corvol MD)

Correspondence to: Dr Emma Baker, Department of Pharmacology, St George's Hospital Medical School, London SW17 0RE, UK (e-mail: ebaker@sghms.ac.uk)

Introduction

The importance of genetic predisposition in essential hypertension has long been established. In rat models of inherited hypertension, kidney cross-transplantation has shown that the kidney carries the genetic defect for high blood pressure which appears to be expressed as a difficulty in excretion of sodium.[1] Circumstantial evidence suggests this may also be true in essential hypertension in human beings.[2] The rare genetic causes of human hypertension all involve increased renal tubular sodium absorption, either indirectly through excess mineralocorticoid activity or directly as in Liddle's syndrome.[3] Liddle's syndrome is caused by mutations of subunits of the epithelial sodium channel that result in increased sodium-channel activity in the distal renal tubule with excess sodium reabsorption.[4,5] This sodium retention causes the high blood pressure and the characteristic suppression of the renin-angiotensin system seen in Liddle's syndrome.[6] High blood pressure in these patients responds well to reduction of salt intake or to amiloride, which acts specifically to reduce the activity of the abnormal channels.[6]

The clinical features of Liddle's syndrome overlap with those of some patients with essential hypertension. In particular, black patients with hypertension are known to be sensitive to changes in salt intake and have low plasma renin activity. Mutations in sodium-channel-subunit genes have been identified in a few patients with essential hypertension and almost all of them in patients of African descent.[7] These mutations affect the same region of the sodium channel as occurs in Liddle's syndrome but differ as they result in a single aminoacid change rather than major truncation of the subunit. When the point mutations found in patients with essential hypertension are expressed in Xenopus oocytes most of them cause a non-significant increase in activity of sodium channels[7] compared with what is observed when the mutations responsible for Liddle's syndrome are expressed.[8] However, sodium-channel activity is increased in lymphocytes from patients with the threonine 594 methionine (T594M) point mutation of the sodium channel β subunit.[9] Therefore, it is possible that this sodium-channel mutation in patients with essential hypertension could contribute to the rise in blood pressure by increasing renal tubular sodium reabsorption.

As yet, it is unknown whether these mutations occur more often in people with hypertension. Therefore we examined the frequency of the T594M point mutation, the most commonly identified sodium-channel mutation,[7,9] to see if it occurred more frequently in hypertensive than in normotensive black people.

Methods and participants

We did a case-control study of hypertensive and normotensive black individuals. Cases were taken from a group of unselected referrals to a hypertension clinic by local general practitioners (GPs) and comprised black patients with high blood pressure attending the clinic for the first time between February, 1995,

and August, 1996, inclusively. Blood was collected from all cases during this time and a random number of samples were analysed. Controls were taken from a population-based, cross-sectional survey of the local population aged 40–59 years done in the same area as the patients referred by GPs (The Wandsworth Heart and Stroke Study).[10] They were randomly selected from GP age-sex registers as described elsewhere.[10] Ethnic origin was defined with a combination of the participant's place of birth and parents' place of birth. All control participants and most hypertensive participants were first generation immigrants to the UK.

All participants rested in a supine position for 5 min after which blood-pressure recordings were done in triplicate with a semi-automated ultrasound sphygmomanometer,[11] with the appropriate cuff size according to the recommendations of the British Hypertension Society.[12] Hypertensive individuals were either on treatment for their high blood pressure or, if not, had a consistent supine systolic blood pressure of more than 140 mm Hg or diastolic blood pressure of more than 90 mm Hg and were considered after clinical and biochemical investigation to have essential hypertension. Normotensive individuals had a supine systolic blood pressure of 140 mm Hg or less and a diastolic blood pressure of 90 mm Hg or less. 206 black participants with essential hypertension (80 men, 126 women; mean age 48·0 [SD 11·8] years) and 142 black control individuals (61 men, 81 women; mean age 48·7 [7·4] years) were studied. Informed consent was obtained from participants before entry into the study, which was approved by the local research ethics committee.

Clinical assessment

Control participants had blood taken for measurement of serum concentrations of sodium and potassium, plasma-renin activity, and aldosterone concentration. The participants also collected their urine for 24 h for estimation of urinary sodium excretion.

All patients with high blood pressure had routine investigations. For many of the patients who were not on medication at the time of assesment, plasma renin activity, aldosterone, and 24 h urinary sodium excretion were measured.

In participants who were subsequently identified as possessing the T594M variant, further clinical assessment was done. Where applicable blood-pressure treatment was stopped for 2 weeks under careful clinical supervision and then plasma-renin activity and aldosterone and 24 h urinary sodium excretion were measured. Plasma renin activity and aldosterone were measured in all participants in the same laboratory by RIA.[13,14] 24-h urinary sodium was measured at St George's Hospital, London, UK, for all participants. Serum sodium and potassium concentrations in the normotensive participants were measured at the University of Naples, Naples, Italy,[10] and of the hypertensive individuals were measured at St George's Hospital. Both laboratories underwent internal and external quality control. No systematic differences were seen between the measurements of electrolytes between the two laboratories.

DNA extraction and analysis

Genomic DNA was isolated from whole-blood samples. Blood was collected in 10 mL K-edetic acid tubes and kept frozen at −20°C until processed for DNA extraction. The extraction procedure was done with a kit (Nucleon BACC DNA Extraction Kit, Nucleon Biosciences). The DNA yield was about 20 μg/mL of whole blood with an estimated purity of more than 90% (calculated from optical-density readings).

All genotyping was done in duplicate for each individual and the investigator was unaware of sample origin, to prevent observer bias.

PCR

Part of the carboxy-terminal portion of the epithelial sodium channel β subunit was amplified from human genomic DNA with the following primer set: a sense primer (5'-ACCGTG-GCCGAGCTGGTGGAG-3') corresponding to nucleotides 1729–1749, according to Voilley and colleagues,[15] and an antisense primer (5'-CAGTCTTGGCTGCTCAGTGAG-3') corresponding to intronic sequence 1954–1974. 100 ng human genomic DNA was amplified with 15 pmol of each primer, 200 μmol/L deoxyribonucleoside triphosphates (dNTPs), 1·5 mmol/L $MgCl_2$, and 2U AmpliTaq polymerase (Perkin Elmer, Warrington, UK). After an initial denaturation step at 94°C for 5 min, PCR amplification conditions were 94°C (30 s), 67·5°C (90 s), and 72°C (30 s) over 35 cycles followed by a final extension at 72°C for 5 min. Size and quality of PCR products were verified by electophoresis on a 2% agarose gel with ethidium bromide staining.

	Normotensive	Hypertensive
Total screened	142	206
Number on treatment	. .	113
Male:female	61:81	80:126
Ethnic origin		
Black Caribbean	69 (48·6%)	100 (48·6%)
Black African	65 (45·8%)	94 (45·6%)
Black other	8 (5·6%)	12 (5·8%)
Age (years)	48·7 (7·4)	48·0 (11·8)
Weight (kg)	74·7 (11·1)	80·3 (13·8)*
Body-mass index (kg/m²)	27·0 (3·9)	29·2 (4·7)*
Systolic BP (mm Hg)		
Whole group	122·1 (10·1)	161·5 (23·5)
On treatment	. .	163·5 (24·7)
Off treatment	. .	159·0 (21·6)
Diastolic BP (mm Hg)		
Whole group	78·7 (6·8)	98·3 (12·0)
On treatment	. .	97·7 (11·8)
Off treatment	. .	98·9 (12·4)

*p<0·0001 *vs* normotensive individuals. Blood-pressure (BP) values in the hypertensive group are given for the whole group and in those on or off antihypertensive treatment. Group values are means (SD).

Table 1: **Clinical characteristics of individuals screened for T594M mutation**

Analysis for single-strand conformational polymorphism

Genotypes were analysed by a system to detect single-strand conformational polymorphism (SSCP). 4 μL aliquots of each PCR product were denatured by addition of 8 μL denaturing solution (94% formamide, 10 mmol/L NaOH, 0·25% bromophenol blue) and heating to 95°C, followed by rapid cooling on ice. Products were separated at 10°C by electrophoresis at 250 V for 12–19 h on 0·5×mutation detection enhancement (MDE) gels prepared in 0·6×tris-borate/EDTA electrophoresis buffer (TBE) and run in 0·6×TBE running buffer. The DNA was visualised by a silver-staining protocol.[16]

DNA sequencing

DNA from individuals identified as possessing a polymorphism of the sodium channel β subunit was sequenced to identify the mutation. For direct sequencing of PCR-amplified products, the amplified fragments were purified with spin columns. The sequencing reactions were done on double-strand DNA with the same primers as those used for PCR in dye terminator cycle sequencing. Extension products were purified by ethanol precipitation. The reactions were loaded onto an ABI 377 automated sequencer (Applied Biosystems, Foster City, California, USA) and run under standard conditions. DNA sequences were confirmed by sequencing both strands.

Statistical analysis

Group values are given as mean (SD) for data with a normal distribution and as median interquartile range (IQR) for plasma renin activity and aldosterone, which are not normally distributed. Differences between groups were tested with two-sample *t* tests for normally distributed variables. Differences in plasma renin activity and plasma aldosterone concentrations were tested with Mann-Whitney *U* test. Differences in the distribution of the frequencies of the T594M variant between normotensive and hypertensive individuals were tested with the χ^2 test, with Yates' correction. In addition we calculated the odds ratio as an estimate of the relative risk of hypertensive individuals having the mutation compared with normotensive individuals. The Mantel-Haenszel pooled estimate of the odds ratio and 95% CI were used in stratified analysis to control for the effect of sex and for differences in body-mass index (BMI). Exact CIs were calculated in each analysis. Stratification for BMI was done by grouping the

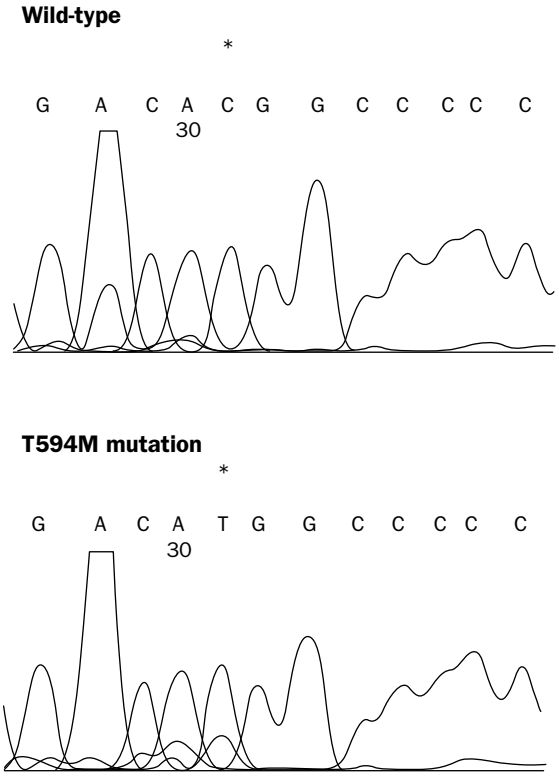

Figure 1: **Sequence comparison of sense strands of DNA from wild-type and T594M mutation**
*Mutation (C→T transition).

participants according to sex-specific tertiles of BMI. Cut-off values for BMI stratification were from a population sample (341 women and 208 men) of black individuals from which the individuals screened for mutations were recruited.[10]

Results

Characteristics of the two groups screened are summarised in table 1. Participants with hypertension were significantly heavier than normotensive participants and had higher BMI. The ethnic mix of participants in hypertensive and normotensive groups was similar, with 48·6% of individuals with hypertension and 48·6% of normotensive individuals having parents from Africa or born of African parents, and 45·6% of participants with hypertension and 45·8% of normotensive participants originating from the Caribbean or born of Caribbean parents.

SSCP analysis identified 17 of the 206 hypertensive participants and three of the 142 normotensive participants in whom there was altered migration of the PCR products, which suggested the presence of the T594M mutation of the sodium-channel β subunit. Duplicate analysis confirmed SSCP typing in all participants. Sequencing of PCR products confirmed that all 20 SSCP variants were heterozygous for the T594M mutation of the sodium channel β subunit (figure 1).

The proportion of participants with the T594M variant was much higher in the hypertensive group (17 [8·3%] of 206) than in the normotensive group (3 [2·1%] of 142) giving a crude odds ratio of 4·17 (95% CI 1·12–18·25); p=0·029. The odds ratio was recalculated after adjustment for sex and BMI (stratified into tertiles) given that the participants with hypertension had a higher BMI and that 18 of 20 participants with the T594M variant were women (table 2). The adjusted odds ratio was of similar magnitude (5·52 [1·40–30·61]; p=0·012) suggesting that the association between the variant and blood-pressure status was independent of sex and BMI.

Clinical characteristics of normotensive and hypertensive participants both with and without the T594M variant are summarised in table 2. In the hypertensive group, there were no significant differences in age, weight, or systolic and diastolic blood pressure between those with or without the variant. Similarly, there did not appear to be any differences in these measurements between the normotensive participants with and without the variant, although the number of normotensive participants with the T594M variant was insufficient for reliable comparison. In those with the T594M variant, the ethnic mix (African or Caribbean origin or descent) was similar to the ethnic mix of the total population.

In the subgroup in whom biochemical and hormonal measurements had been made on no medication, plasma renin activity was significantly lower in hypertensive individuals with the T594M variant (0·19; 0·1–0·3 ng mL^{-1} h^{-1}; n=13) than in hypertensive individuals without the variant (0·45, 0·21–0·88 ng mL^{-1} h^{-1}; n=39, p=0·009, Mann-Whitney *U* test; figure 2). Plasma renin activity was also lower in the three normotensive participants with the T594M variant (medium 0·17 ng mL^{-1} h^{-1}) than in the normotensive participants without the variant (0·39, 0·22–0·67 ng mL^{-1} h^{-1}, n=65). There were no significant differences in serum Na, K, or aldosterone or 24 h urinary Na between hypertensive participants with or without the T594M variant or between normotensive individuals with or without the T594M variant.

Genotype	Normotensive		Hypertensive	
	Wild type (n=139)	T594M (n=3)	Wild type (n=189)	T594M (n=17)
Number on treatment	. .	. .	105	8
Male:female	61:78	0:3	78:111	2:15
Ethnic origin				
Black Caribbean	66	3	93	7
Black African	65	0	84	10
Black other	8	0	12	0
Age (years)	48·6 (7·4)	50·7 (8·4)	47·9 (11·7)	48·5 (12·8)
Weight (kg)	74·7 (11·2)	70·7 (5·5)	80·6 (14·0)	76·9 (11·3)
Body-mass index (kg/m^2)	27·0 (4·0)	27·2 (2·7)	29·2 (4·7)	28·2 (4·0)
Systolic BP (mm Hg)*				
All participants	122·1 (10·1)	119·3 (9·7)	161·0 (23·6)	167·7 (21·6)
On treatment	. .	. .	163·6 (25·2)	163·5 (18·6)
Off treatment	. .	. .	157·7 (21·0)	171·5 (24·5)
Diastolic BP (mm Hg)*				
All participants	78·8 (6·8)	75·2 (1·2)	98·3 (12·1)	98·2 (11·9)
On treatment	. .	. .	97·8 (11·7)	96·5 (12·9)
Off treatment	. .	. .	98·8 (12·5)	99·8 (11·6)
Serum Na (mmol/L)†	140·0 (2·6) (n=139)	139·3 (2·4) (n=3)	139·9 (2·6) (n=72)	140·5 (2·7) (n=13)
Serum K (mmol/L)†	4·2 (0·3) (n=14)	4·4 (0·4) (n=3)	4·2 (0·4) (n=72)	4·1 (0·5) (n=13)
Urine Na (mmol/24 h)†	160·2 (73·4) (n=92)	159·1 (36·0) (n=3)	154·2 (79·3) (n=59)	131·7 (68·4) (n=12)
Aldosterone (pmol/L)†‡	278 (207·5–384·5) (n=129)	187 (n=3)	386·5 (220–510) (n=52)	428 (320·5–569·5) (n=13)

Group values are means (SD) for normally distributed data.
*BP values in the hypertensive group are given for the whole group and in those on or off antihypertensive treatment. †Serum and urine biochemistry and aldosterone are given for those participants in whom measurements were made while they were on no medication. The number of individuals in whom each measurement was obtained is given below the mean value. ‡Median and IQR range.

Table 2: **Comparison of clinical characteristics of black individuals with and without the T594M variant**

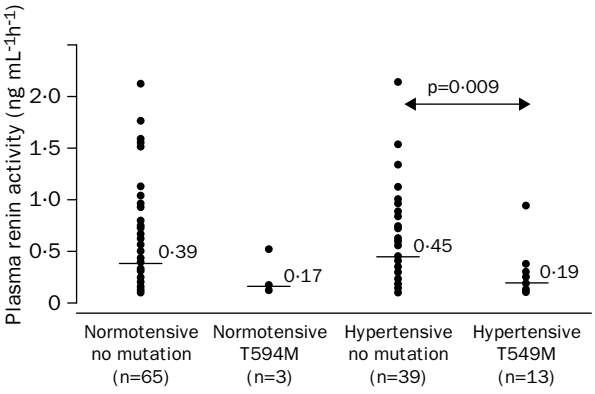

Figure 2: **Plasma renin activity in black normotensive and hypertensive individuals with and without T594M mutation**
Individual values and median are shown.

Discussion

In this case-control study of black London residents we have shown that the T594M variant of the β subunit of the epithelial sodium channel occurs significantly more frequently in individuals with hypertension (8·3%) than in those without (2·1%, p=0·029). The T594M variant in the individuals studied is associated with hypertension and could potentially be responsible for the development of high blood pressure in affected individuals.

The T594M mutation affects the last exon of the sodium channel β subunit and results in a single aminoacid change with substitution of methionine for threonine.[7] The threonine residue at position 594 in the β subunit is a potential target for phosphorylation by protein kinase C which inhibits sodium-channel activity.[9] The T594M mutation could cause the channel to become resistant to the negative regulatory effects of protein kinase C. Whole cell patch-clamp measurements of lymphocytes from individuals with and without the T594M mutation have suggested that this is indeed the case.[9] Lymphocytes with the T594M variant show inward amiloride-sensitive sodium current, which has an enhanced response to cAMP compared with that seen in controls, possibly due to the loss of suppressive effect of protein kinase C.[9] Conversely, the T594M mutation did not induce a significant increase in sodium transport when expressed in xenopus oocytes,[7] which could reflect only a mild effect in this expression system. Evidence from lymphocyte studies suggests that the T594M mutation may increase sodium-channel activity by causing affected channels to become insensitive to negative regulation. If the T594M mutation also has this effect on sodium channels in the renal tubules, then it could contribute to the development of high blood pressure in affected individuals by reducing renal sodium excretion and causing sodium retention.

In hypertensive individuals we found the plasma renin activity to be significantly lower in patients with the T594M variant than in individuals without the variant. This suggests that possession of the T594M variant is indeed associated with sodium retention and is consistent with a role for the T594M mutation in the development of high blood pressure. However, plasma-renin activity was suppressed both in 13 hypertensive individuals and in the three normotensive individuals with the T594M variant. The effects of the T594M variant on blood pressure may be modulated by other genetic or environmental factors. Plasma aldosterone concentrations were not significantly lower in hypertensive individuals with the T594M variant compared with individuals with hypertension but without the variant, although in the three normotensive individuals with the T594M variant aldosterone concentrations were low. The degree of suppression of aldosterone in response to increased sodium reabsorption in patients with the T594M variant may be one additional factor which determines the blood pressure in these patients.

In our study blood-pressure values and serum biochemistry in individuals with the T594M variant were not significantly different from those of unaffected individuals with hypertension. It is important to point out, however, that even in patients with Liddle's syndrome, where a more radical mutation leads to a clear increase in sodium-channel activity, blood pressure, and serum potassium may not differ strikingly from those with essential hypertension. For instance, in a recent study of Liddle's syndrome, 18 affected family members related to the original Liddle's proband were characterised.[17] At the time of diagnosis the mean blood pressure in these individuals was only 148 (5)/97 (2) mm Hg and serum potassium was 3·6 (0·1) mmol/L.

If the T594M mutation does indeed predispose to high blood pressure, a family history of hypertension might be expected in individuals with the T594M variant. Participants in our study with the T594M variant did have a positive family history of hypertension with 53·3% reporting at least one parent with high blood pressure. This is likely to be an underestimate of the true parental prevalence of hypertension because family history is subject to recall bias. Under-reporting of parental hypertension occurs because high blood pressure is undetected in around 25% of all those with hypertension.[10] In addition, many of the participants in our study were first-generation immigrants, which may have further complicated their knowledge of parental blood-pressure status. Ideally, blood pressure status of both parents should be available to define family history precisely, but this was not possible in the participants in our study. Reported family history of hypertension did not differ significantly between hypertensive individuals with and without the T594M variant. However, hypertension is a familial trait and would be expected to be common in parents of both these groups.

In any case-control study it is necessary to consider whether selection of cases or controls or ascertainment of exposures may have biased the results. The controls were randomly selected from general practices in the same area as that served by the hypertension clinic. The most likely selection bias in such a situation would arise if the prevalence of the variant differed between black groups in the area and those with a higher prevalence were more likely to be seen in the hypertension clinic (eg, for cultural reasons). This seems unlikely since the association between the variant and hypertension is seen in both blacks of Caribbean and African origin. Where the exposure is genetic, bias in assessment of exposure can be ruled out. Experimental bias was removed by masking the experimenter to the case or control origin of samples during assessment of T594M status.

The effect of sex and BMI (stratified into tertiles) on the association between the variant and blood-pressure status was checked by calculation of an odds ratio adjusted for these variables. The adjusted odds ratio was 5·52 (CI 1·40–30·61), suggesting that the association

between the variant and blood-pressure status was not confounded by BMI or sex of the participants. It is not clear why such a high proportion of the participants with the T594M variant were women. This finding may reflect early death of affected men, although this is unlikely because the average age of the hypertensive participants is 48 (11·8) years and of the normotensive participants being 48·7 (7·4) years. It may also reflect different expression in women in the presence of modulating environmental factors (eg, salt intake, obesity). Black women tend to be more obese than black men.[10] However, the prevalence of hypertension in black people does not differ between men and women and the T594M variant appears to be associated with lower rather than higher body weight.

The T594M mutation has not so far been identified in non-black individuals. A study of 250 unrelated individuals with unknown blood pressure in America did not identify any sodium-channel mutations.[4] In a study of 90 hypertensive and 51 normotensive Japanese people, the investigators did not find any missense mutations by direct screening of the last exon of the β subunit using PCR amplification and sequencing techniques.[18] Other studies of black people have not shown an association between abnormalities of the sodium-channel β subunit and high blood pressure. Linkage analysis of 63 affected sibling pairs of West African origin from St Vincent and the Grenadines failed to show an association of the α, β, or γ subunit genes with hypertension.[19] However, the power of this study was far too weak to exclude a role for sodium-channel abnormalities in the development of high blood pressure in this population. In a study of Americans, the T594M mutation has been shown to occur in black people but not white people. However, unlike our study, there was no difference in the prevalence of the T594M mutation in black people with (7 [5·6%] of 126) and without (7 [6·7%] of 105) hypertension. The difference in the distribution of the mutation in hypertensive and normotensive groups between the two studies may reflect differences in other genetic factors, given the more mixed genetic ethnicity of African Americans compared to first-generation black immigrants to the UK. It may also reflect differences in environmental exposure, particularly differences in sodium or potassium intake between individuals and between populations from whom participants were recruited.

Our results suggest that 8% of black people who live in London and have hypertension possess a single sodium-channel subunit mutant allele, which may contribute to their high blood pressure. This does not exclude the possibility that other sodium-channel mutations may also play an important part in hypertension in other black people. If the role of sodium-channel mutations in the development of hypertension is confirmed by further studies then they will comprise the most common secondary cause of hypertension in black people identified so far. This should allow a more rational approach to treatment of high blood pressure in affected individuals.

Contributors

E Baker, Y Dong, G Sagnella, F Cappuccio, D Cook, A Persu, P Corvol, X Jeunemaitre, N Carter, and G MacGregor were responsible for the conception and design of the study. E Baker, Y Dong, G Sagnella, M Rothwell, A Onipinla, N Markandu, F Cappuccio, and D Cook collected the data and all authors took part in data analysis and interpretation. E Baker, Y Dong, G Sagnella, F Cappuccio, D Cook, and G McGregor drafted the article and all authors took part in its revision. All authors gave their approval for the final version of the article.

Acknowledgments

This study was supported by the British Heart Foundation. The Wandsworth Heart and Stroke Study was supported by the Wandsworth Health Authority, the South West Thames Region, the NHS Research and Development, the British Heart Foundation, and the British Diabetic Association. AP was partly supported by the Société Française d' Hypertension Arterielle. We thank Andrew Crosby, Steve Jeffrey, Patrick Tarpey, and Chrissie Fenske of the Medical Genetics Unit for their scientific contributions. We also thank Alison Blackwood, Liz Folkerd, and Michelle Miller for the assays of plasma renin activity and plasma aldosterone.

References

1 deWardener HE, MacGregor GA. The natriuretic hormone and essential hypertension. *Lancet* 1982; i: 1450–54.
2 Curtis JJ, Luke RG, Dustan HP, et al. Remission of essential hypertension after renal transplantation. *N Engl J Med* 1983; **309:** 1009–15.
3 Lifton RP. Molecular genetics of human blood pressure variation. *Science* 1996; **272:** 676–80.
4 Shimkets RA, Warnock DG, Bositis CM, et al. Liddle's syndrome: heritable human hypertension caused by mutations in the β subunit of the epithelial sodium channel. *Cell* 1994; **79:** 407–14.
5 Hansson JH, Nelson-Williams C, Suzuki H, et al. Hypertension caused by a truncated epithelial sodium channel γ subunit: genetic heterogeneity of Liddle's syndrome. *Nat Genet* 1995; **11:** 76–82.
6 Liddle GW, Bledsoe T, Coppage WS Jr. A familial renal disorder simultating primary aldosteronism but with negligible aldosterone secretion. *Trans Assoc Am Phys* 1963; **76:** 199–213.
7 Persu A, Barbry P, Bassilana F, et al. Genetic analysis of the β subunit of the epithelial Na^+ channel in essential hypertension. *Hypertension* 1998; (in press).
8 Schild L, Canessa CM, Shimkets RA, Gautschi I, Lifton RP, Rossier BC. A mutation in the epithelial sodium channel causing Liddle disease increases sodium channel activity in the *Xenopus laevis* oocyte expression system. *Proc Natl Acad Sci USA* 1995; **92:** 5699–703.
9 Su YR, Rutkowski MP, Klanke CA, et al. A novel variant of the β-subunit of the amiloride-sensitive sodium channel in African Americans. *J Am Soc Nephrol* 1996; **7:** 2543–49.
10 Cappuccio FP, Cook DG, Atkinson RW, Strazzullo P. Prevalence, detection and management of cardiovascular risk factors in different ethnic groups in South London. *Heart* 1997; **78:** 555–63.
11 George CF, Lewis PJ, Petrie A. Clinical experience with use of ultrasound sphygmomanometer. *Br Heart J* 1975; **37:** 804–07.
12 Technique of blood pressure measurement. *J Hypertens* 1985; **3:** 293.
13 Roulston JE, MacGregor GA. Measurements of plasma renin activity by radioimmunoassay after prolonged cold storage. *Clin Chem Acta* 1978; **88:** 45–48.
14 James VHT, Wilson GA. Assays of drugs and other trace compounds in biological fluids. In: Reid E, ed. Methodological Development in Biochemistry, vol 5. Amsterdam: Elsevier, 1976: 149–58.
15 Voilley N, Bassilana F, Mignon C, et al. Cloning, chromosomal localisation and physical linkage of the β and γ subunits (SCNN1B and SCNN1G) of the human epithelial amiloride-sensitive sodium channel. *Genomics* 1995; **28** 560–65.
16 Sammons DW, Adams LD, Nishizawe EE. Ultrasensitive silver-based colour staining of polypeptides in polyacrylamide gels. *Electrophoresis* 1991; **2:** 135–41.
17 Botero-Velez M, Curtis JJ, Warnock DG. Liddle's syndrome revisited—a disorder of sodium reabsorption in the distal tuble. *N Engl J Med* 1994; **330:** 178–81.
18 Chang H, Fujita T. Lack of mutations in epithelial sodium channel β subunit gene in human subjects with hypertension. *J Hypertens* 1996; **14:** 1417–19.
19 Munroe PB, Strautnieks SS, Farrall M, et al. Investigation of the epithelial sodium channel gene (hENaC) as a candidate gene for essential hypertension in African Caribbeans. *Am J Hypertens* 1997; **10:** 5A.