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


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.4 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.5

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.6 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 channels7 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,10,11 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). They were randomly selected from GP age-sex registers as described elsewhere. 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, with the appropriate cuff size according to the recommendations of the British Hypertension Society. 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 participants had a consistent supine systolic blood pressure of more than 140 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. All control participants and most hypertensive participants were first-generation immigrants to the UK.

All genotyping was done in duplicate for each individual and the T594M mutation was confirmed by DNA sequencing. The reactions were loaded onto an ABI 377 automated sequencer (Applied Biosystems, Foster City, CA, USA). After an initial denaturation step at 94ºC for 3 min, PCR amplification conditions were 94ºC (30 s), 67·5ºC (90 s), and 72ºC (30 s) over 35 cycles followed by a final extension at 72ºC for 5 min. Size and quality of PCR products were verified by electrophoresis on a 2% agarose gel with ethidium bromide staining. The DNA was visualised by a silver-staining protocol.

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 χ² 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

<table>
<thead>
<tr>
<th>Ethnic Origin</th>
<th>Normotensive</th>
<th>Hypertensive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black African</td>
<td>65 (45·8%)</td>
<td>54 (45·6%)</td>
</tr>
<tr>
<td>Black Caribbean</td>
<td>69 (48·6%)</td>
<td>100 (48·6%)</td>
</tr>
<tr>
<td>Black other</td>
<td>6 (5·6%)</td>
<td>12 (5·8%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48·7 (7·4)</td>
<td>48·0 (11·8)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74·7 (11·4)</td>
<td>80·3 (13·8)*</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27·0 (3·9)</td>
<td>29·2 (4·7)*</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole group</td>
<td>122·1 (10·1)</td>
<td>161·5 (23·5)</td>
</tr>
<tr>
<td>Off treatment</td>
<td></td>
<td>163·5 (24·7)</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole group</td>
<td>78·7 (6·9)</td>
<td>98·3 (12·0)</td>
</tr>
<tr>
<td>Off treatment</td>
<td></td>
<td>97·7 (11·8)</td>
</tr>
</tbody>
</table>

*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

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18 of 20 participants with the T594M variant that the participants with hypertension had a higher BMI adjustment for sex and BMI (stratified into tertiles) given p=0.029. The odds ratio was recalculated after giving a crude odds ratio of 4.17 (95% CI 1.12–18.25); was much higher in the hypertensive group (17 [8.3%] of all 20 SSCP variants were heterozygous for the T594M mutation of the sodium-channel subunit. Sequencing of PCR products confirmed that participants in whom there was altered migration of the wild-type and T594M mutation 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.

**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 46.8% 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 born of Caribbean or born of 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.

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

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Normotensive</th>
<th>Hypertensive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>Wild type</td>
<td>Hypertensive</td>
</tr>
<tr>
<td>(n=139)</td>
<td>(n=189)</td>
<td>(n=17)</td>
</tr>
<tr>
<td>Number on treatment</td>
<td>. . . .</td>
<td>105</td>
</tr>
<tr>
<td>Male:female</td>
<td>61.78:38.3</td>
<td>76:111</td>
</tr>
<tr>
<td>Ethnic origin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caribbean</td>
<td>66</td>
<td>3</td>
</tr>
<tr>
<td>Black African</td>
<td>65</td>
<td>0</td>
</tr>
<tr>
<td>Black other</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48.6 (7.4)</td>
<td>50.7 (8.4)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.7 (11.2)</td>
<td>70.7 (5.5)</td>
</tr>
<tr>
<td>Body-mass index</td>
<td>27.9 (5.0)</td>
<td>27.2 (2.7)</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)*</td>
<td>. . . .</td>
<td>157.7 (21.0)</td>
</tr>
<tr>
<td>All participants</td>
<td>122.1 (10.1)</td>
<td>159.3 (9.7)</td>
</tr>
<tr>
<td>On treatment</td>
<td>. . . .</td>
<td>163.6 (25.2)</td>
</tr>
<tr>
<td>Off treatment</td>
<td>. . . .</td>
<td>157.7 (21.0)</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)*</td>
<td>. . . .</td>
<td>98.3 (12.1)</td>
</tr>
<tr>
<td>All participants</td>
<td>78.8 (6.8)</td>
<td>75.2 (1.2)</td>
</tr>
<tr>
<td>On treatment</td>
<td>. . . .</td>
<td>97.8 (11.7)</td>
</tr>
<tr>
<td>Off treatment</td>
<td>. . . .</td>
<td>98.8 (12.5)</td>
</tr>
<tr>
<td>Serum Na (mmol/L)</td>
<td>140.0 (2.6)</td>
<td>139.3 (2.4)</td>
</tr>
<tr>
<td>(n=139)</td>
<td>(n=3)</td>
<td>(n=72)</td>
</tr>
<tr>
<td>Serum K (mmol/L)</td>
<td>4.2 (0.3)</td>
<td>4.4 (0.4)</td>
</tr>
<tr>
<td>(n=134)</td>
<td>(n=3)</td>
<td>(n=72)</td>
</tr>
<tr>
<td>Urine Na (mmol/L)</td>
<td>160.2 (7.3)</td>
<td>159.1 (6.0)</td>
</tr>
<tr>
<td>(n=92)</td>
<td>(n=3)</td>
<td>(n=52)</td>
</tr>
<tr>
<td>Aldosterone (pmol/L)</td>
<td>384.5 (51.0)</td>
<td>386.3 (200.0)</td>
</tr>
<tr>
<td>(n=129)</td>
<td>(n=52)</td>
<td>(n=13)</td>
</tr>
</tbody>
</table>

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.
subunit is a potential target for phosphorylation by case.9 Lymphocytes with the T594M variant show inward resistance to the negative regulatory effects of protein threonine.7 The threonine residue at position 594 in the 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 epithelial sodium channel occurs significantly more frequently than 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 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.7 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.7 Conversely, the T594M mutation did not induce a significant increase in sodium transport when expressed in xenopus oocytes,1 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 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.15 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.14 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 and 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
coufounded 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 normotenive 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.1 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.14 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.15 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 America,
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 Peruzzi, 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 O'Ruapulu, 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 MacGregor drafted the article and all authors took part in its revision. All
authors gave their approval for the final version of the article.

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scientific contributions. We also thank Alison Blackwood, Liz Folkard,
and Michelle Miller for the assays of plasma renin activity and plasma
aldosterone.

References
1 deWardener HE, MacGregor GA. The natriuretic hormone and
3 Lifton RP. Molecular genetics of human blood pressure variation.
4 Shimkets RA, Warnock DG, Bostis CM, et al. Liddle’s syndrome:
heritable human hypertension caused by mutations in the β subunit
by a truncated epithelial sodium channel γ subunit: genetic
6 Liddle GW, Bledsoe T, Coppage WS Jr. A familial renal disorder
simulating primary aldosteronism but with negligible aldosterone
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
9 Su YR, Rütkowski MP, Klánke CA, et al. A novel variant of the
β-subunit of the amiloride-sensitive sodium channel in African
10 Cappuccio FP, Cook DG, Atkinson RW, Strazzullo P. Prevalence,
detection and management of cardiovascular risk factors in different
11 George CF, Lewis PJ, Petrie A. Clinical experience with use of
13 Roulston JE, MacGregor GA. Measurements of plasma renin activity
by radioimmunoassay after prolonged cold storage. Clin Chem Acta
14 James VHT, Wilson GA. Assays of drugs and other trace compounds
in biological fluids. In: Reid E, ed. Methodological Development in
localisation and physical linkage of the β and γ subunits (SCNN1B
and SCNN1G) of the human epithelial amiloride-sensitive sodium
16 Summons DW, Adams LD, Nishizawa EE. Ultrasonic silver-based
colour staining of polypeptides in polyacrylamide gels.
17 Botero-Velez M, Curtis JJ, Warnock DG. Liddle’s syndrome
revisited—a disorder of sodium reabsorption in the distal tubule.
18 Chang H, Fujita T. Lack of mutations in epithelial sodium channel β
14: 1417–19.
19 Munroe PB, Strautnieks SS, Farrell M, et al. Investigation of the
epithelial sodium channel gene (hENaC) as a candidate gene for
10: 5A.