

# Association Between the C825T Polymorphism of the G Protein $\beta$ 3-Subunit Gene and Hypertension in Blacks

Yanbin Dong, Haidong Zhu, Giuseppe A. Sagnella, Nicholas D. Carter,  
Derek G. Cook, Francesco P. Cappuccio

**Abstract**—A polymorphism (C825T) of the G protein  $\beta$ 3-subunit gene has been associated with low renin hypertension in whites. The aim of this study was to examine the C825T polymorphism in relation to hypertension in a population-based study of black people of African origin who have high prevalence of low renin, salt-sensitive hypertension. A total of 428 men and women, aged 40 to 59 years (270 Caribbeans and 158 West Africans), who took part in a population-based survey were studied. All were blacks and first-generation immigrants. The C825T polymorphism was detected by polymerase chain reaction followed by restriction-enzyme digestion. The prevalence of hypertension (supine blood pressures  $\geq 160$  systolic and/or 95 mm Hg diastolic or on drug therapy) was 43%. The distribution of the genotypes (CC, CT, and TT) was in Hardy-Weinberg equilibrium with observed frequencies of 4.0% (n=17), 33.6% (n=144), and 62.4% (n=267), respectively. Allele frequencies were 20.8% for C and 79.2% for T. No difference was detected between Caribbeans and West Africans. A 3-fold higher risk of hypertension was found among the carriers of the T variant both as heterozygotes (odds ratio [OR], 3.43 [95% CI, 0.94 to 12.4]) and homozygotes (OR, 3.87 [95% CI, 1.09 to 13.8]). The estimate of effect and the blood pressure values in the groups carrying the T variant suggested a dominant model for the T allele. This was confirmed by a significant association between the T allele and hypertension (OR, 3.71 [95% CI, 1.05 to 13.1]), even when adjusted for age, sex, and body mass index (OR, 4.14 [95% CI, 1.11 to 15.4]). The study shows, for the first time, a high frequency of the 825T allele in black people, and it provides evidence that the T allele may be a susceptibility factor for the development of hypertension in blacks. Given the high frequency of the T allele, even a 2-fold increased risk of hypertension among the carriers of the T allele might account for 44% of the cases of hypertension in blacks. (*Hypertension*. 1999;34:1193-1196.)

**Key Words:** hypertension, genetic ■ blacks ■ G protein ■ polymorphism ■ epidemiology

GTP binding proteins (G proteins) mediate the functional responses of numerous agonists.<sup>1</sup> Hence, variations within genes coding for these proteins may be of significance in cardiovascular disease.

Polymorphism C $\rightarrow$ T at nucleotide 825 in exon 10 of the  $\beta$ 3-subunit of pertussis toxin-sensitive G<sub>i</sub>-type protein has recently been identified. A significantly higher frequency of the T allele has been reported in subjects with essential hypertension compared with unselected normotensive control subjects of European origin in 3 independent studies<sup>2-4</sup> but not in a fourth study.<sup>5</sup>

The recombinant, mutated G protein  $\beta$ 3-subunit, coexpressed with G $\alpha$ i2 and G $\gamma$ 5 subunits in cell lines from hypertensive patients and in transfected insect cells, increases sensitivity to agonists that stimulate intracellular signaling through pertussis toxin-sensitive G proteins.<sup>2</sup> The mechanism whereby the 825T variant may lead to hypertension in humans remains unknown, but it may involve increased Na<sup>+</sup>-H<sup>+</sup> exchanger activity.<sup>2</sup> Increased activity of this ex-

changer provides several mechanisms of potential relevance to the development of hypertension,<sup>6</sup> including enhancement of renal tubular sodium reabsorption that leads to an increase in extracellular volume. This mechanism may play an important role, given that the 825T allele is associated with lower plasma renin activity and an elevated aldosterone-renin ratio.<sup>7</sup>

Therefore, the possible participation of the 825T mutation may be even more relevant to hypertension in black people of African descent, because their hypertension is characterized by low plasma renin activity, which suggests a corrected state of volume expansion.<sup>2,8-9</sup> The aim of the present study was to examine the C825T polymorphism of the G protein  $\beta$ 3-subunit in relation to hypertension in black people of African origin in a population-based study.

## Methods

A population-based sample of 459 men and women, aged 40 to 59 years, of black African descent (Caribbeans and West Africans) was obtained from stratified random sampling of lists from general

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From the Blood Pressure Unit (Y.D., H.Z., G.A.S., F.P.C.), Department of Medicine, the Medical Genetics Unit (Y.D., N.D.C.), Department of Child Health, and the Department of Public Health Sciences (D.G.C.), St George's Hospital Medical School, London, UK.

Correspondence to Dr G.A. Sagnella (Molecular Biology) or Dr F.P. Cappuccio (Epidemiology), Blood Pressure Unit, Department of Medicine, St George's Hospital Medical School, Cranmer Terrace, SW17 0RE London, UK. E-mail g.sagnella@sghms.ac.uk or f.cappuccio@sghms.ac.uk

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TABLE 1. Characteristics of Participants According to C825T Genotype

Measurement	Genotype			P*
	CC	CT	TT	
Body mass index, kg/m <sup>2</sup>	29.7±1.0 (17)	28.2±0.4 (144)	28.4±0.3 (265)	0.37
Blood pressure, mm Hg				
Systolic	130.2±4.7 (17)	135.6±1.6 (144)	135.2±1.2 (267)	0.55
Diastolic	83.5±2.5 (17)	85.8±0.8 (144)	86.8±0.6 (144)	0.29
On treatment, n (%)	3 (17.6)	47 (32.6)	92 (34.5)	
Serum				
Sodium, mmol/L	139.6±0.7 (17)	139.5±0.2 (140)	140.0±0.2 (265)	0.22
Potassium, mmol/L	4.22±0.07 (17)	4.16±0.02 (140)	4.13±0.02 (265)	0.40
Creatinine, μmol/L	88.7±3.6 (17)	92.3±1.2 (140)	92.0±0.9 (265)	0.63
Plasma aldosterone, pmol/L	311±45 (17)	367±16 (138)	336±11 (261)	0.21

Characteristics are adjusted for age and sex. All values are mean±SE unless otherwise indicated. Values in parentheses indicate number of subjects.

\*P for heterogeneity by ANCOVA.

practices in South London as previously described.<sup>9-10</sup> Participants were all first-generation immigrants. Ethnic group was recorded at the time of interview.<sup>9</sup> All participants gave informed consent to participate in the study, which was approved by the local ethics committee. Participants attended a dedicated screening unit at St. George's Hospital between 8 AM and 12 PM after an overnight fast. Anthropometry and blood pressure measurements were performed as previously described.<sup>9-11</sup> Details of blood collections for DNA, routine serum electrolytes, plasma aldosterone, and urine collections were also as described.<sup>9-11</sup> Hypertension was defined as supine systolic blood pressure ≥160 and/or diastolic blood pressure ≥95 mm Hg or being on antihypertensive treatment.

### Genetic Analysis

Genomic DNA was isolated from whole blood using Nucleon BACC DNA extraction kit.<sup>11</sup> The C825T polymorphism was detected by PCR followed by *Bse*DI (MBI Fermentas) restriction-enzyme digestion as described previously, with minor modifications;<sup>2</sup> products were separated on 2% agarose gels and visualized under UV light by ethidium bromide staining. Genotype was confirmed by direct sequence analysis with the use of a dye terminator kit on an ABI 377 automated sequencer. To prevent observer bias, the investigator was unaware of sample origin and all gels were cross checked by a separate individual. Valid genotyping was obtained in a total of 428 black individuals (93.2%). The characteristics of those not genotyped (6.8%) did not differ from the rest of the population (data not shown).

### Statistical Analysis

The calculation of allele frequency to test for Hardy-Weinberg equilibrium was performed by use of standard methods.<sup>12</sup> Associations between hypertension and genotype were tested with  $\chi^2$  tests. Multiple logistic regression was used to test the effect of genotype on the likelihood of hypertension while controlling for confounding factors. Accordingly, the association between presence of hypertension and genotype was tested by calculating odds ratios (ORs) under a dominant model with scores of 0 for CC and 1 for the TC and the TT genotypes combined. When appropriate, 1-way ANCOVA was used for comparisons of group means adjusted for confounders. To study the attributable risk of hypertension to the presence of the T variant, population-attributable risk percent (PAR%) was estimated as follows:  $PAR\% = \{[(Prev_E)(OR-1)]/1 + [(Prev_E)(OR-1)]\} \times 100$ , where  $Prev_E$  is the prevalence of the exposure (T allele frequency) and OR is the

estimated OR of the association between the T allele and the presence of hypertension.<sup>13</sup> Group values are given as mean±SE.

### Results

Frequencies of genotypes CC, CT, and TT in the whole group were in Hardy-Weinberg equilibrium with observed frequencies of 4.0% (n=17), 33.6% (n=144), and 62.4% (n=267), respectively, and frequencies of 20.8% for C and 79.2% for T allele. No significant difference existed in frequencies between West Africans (n=158; 2.5%, 30.4%, and 67.1% for CC, CT, and TT, respectively) or Caribbeans (n=270; 4.8%, 35.6%, and 59.6% for CC, CT, and TT, respectively). No significant differences existed in age- and sex-adjusted characteristics by genotype (Table 1). A higher prevalence of hypertension was seen among the carriers of the T variant (both as heterozygotes and homozygotes) compared with the CC genotype, even after adjustment for age, sex, and body mass index ( $\chi^2$  for trend=2.91;  $P=0.08$ ) (Table 2). Although the association was of borderline significance, the estimate of effect suggested the possibility of a dominant model for the T allele. In addition, the blood pressures (when adjusted for age and sex) tended to be higher in those with the 825T variant (Table 1). Although it was not statistically significant, this tendency toward increases in blood pressure in those with the T allele was also found in the 279 participants who were not on antihypertensive therapy (TT, 138.0±1.5/87.5±0.7 mm Hg and CT, 138.6±1.9/86.5±1.0 mm Hg versus CC, 131.0±5.5/84.7±2.8 mm Hg;  $P$  by ANCOVA=0.43 and 0.49 for systolic and diastolic, respectively). These findings suggested a dominant model for the T allele. Further analyses were then performed by use of logistic regression under an a priori defined dominant model for the T allele. The results confirmed a significant and independent association between the T allele and hypertension (Table 2). No interaction was detected between sex, age, and body mass index. Serum electrolytes and creatinine did not vary by genotype (Table 1). Age- and sex-adjusted

TABLE 2. Association Between C825T Polymorphism and Hypertension

Genotype	Total, n	Hypertensives, n (%)	Normotensives, n (%)	Crude OR (95% CI)	P	Adjusted OR* (95% CI)	P
CC	17	3 (1.6)	14 (5.8)	1.00		1.00	
CT	144	61 (33.0)	83 (34.1)	3.43 (0.94–12.4)	0.061	3.89 (1.01–14.9)	0.047
TT	267	121 (65.4)	146 (60.1)	3.87 (1.09–13.8)	0.037	4.28 (1.14–16.1)	0.031
$\chi^2$ for trend=2.91; $P=0.08$							
Dominant model							
CC	17	3 (1.6)	14 (5.8)	1.00		1.00	
CT+TT	411	182 (98.4)	229 (94.2)	3.71 (1.05–13.1)	0.040	4.14 (1.11–15.4)	0.032
$\chi^2$ (with Yates' correction)=3.70; $P=0.05$							

\*OR adjusted for age, sex, and body mass index.

plasma aldosterone levels were also comparable across genotypes (Table 1). Similar results were found in those not on treatment (data not shown).

### Discussion

This study demonstrates a high frequency of the 825T allele in black people (79.2%; 95% CI, 76.5 to 81.9) compared with that reported in whites elsewhere<sup>2–5</sup> (28.1%; 95% CI, 26.0 to 30.2)<sup>2</sup> and in our own work (28.6%; 95% CI, 22.3 to 34.9 [Y.D., unpublished observation, 1999]). Moreover, the T allele is significantly associated with hypertension (Table 2), which suggests that this genetic variant could be a predisposing factor to hypertension in blacks. The strength of the association between the T allele and hypertension is compatible with that reported in 3 separate studies in whites (OR, 1.44 [95% CI, 1.09 to 1.90]<sup>2</sup>; OR, 1.5 [95% CI, 1.1 to 2.2]<sup>3</sup>; and OR, 2.3 [95% CI, 1.7 to 3.3]).<sup>4</sup> High blood pressure is a serious problem in African and Caribbean people living in the UK and affects almost 1 in 2 by the age of 50.<sup>10,14</sup> Given the high prevalence of the T allele in blacks, an OR of even 2 would explain a high proportion of the hypertension occurring in blacks. For example, if the T allele is causative and this doubles the risk of hypertension, given a frequency of the T allele of 0.80, the population-attributable risk is 44%, which indicates the proportion of hypertension in blacks attributable to this genetic variant.

Our study has several important aspects in addressing genetic variations within a population. It investigates for the first time the frequency of the C825T polymorphism in a population-based sample of black people of African origin. It examines first-generation immigrants with both parents born in the country of origin and belonging to the same ethnic group, thus reducing the potential effect arising from an unknown degree of admixture. The sample is from the same geographic area, thereby mitigating the potential effects of differences in environmental background.

G protein-containing subunits are involved in several types of transduction pathways.<sup>1</sup> Hence, defects in G protein signaling could lead to hypertension through a multiplicity of mechanisms that operate at distinct levels. Expression of the T allele leads to an in-frame deletion of the G $\beta$ 3 protein,

which is associated with enhanced sensitivity of G $\beta$  proteins to receptor activation. Therefore, a higher blood pressure could arise from increased sensitivity to vasoactive pressor hormones known to transmit their signals through G $\beta$ 3 proteins.<sup>15</sup> Alternatively, an increase in renal sodium reabsorption through increased activity of the renal Na<sup>+</sup>-H<sup>+</sup> exchanger could mediate the rise in blood pressure.<sup>2</sup> In our study, we did not detect any difference in plasma electrolytes and aldosterone according to C825T genotype. However, plasma aldosterone is not a sensitive marker of volume status and the effect on sodium balance remains a possibility.

In summary, this study shows a high frequency of the 825T allele in black people and provides preliminary evidence that the 825T allele may be a significant susceptibility factor for the development of hypertension in blacks. However, given the high frequency of the T allele, further work is needed to determine more precisely its effect on hypertension in black people. Although these findings clearly require independent confirmation, together with previous work,<sup>16</sup> they highlight the possible contribution of variants in genes regulating renal sodium handling in the development of hypertension in blacks.

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