

A population study of ethnic variations in the angiotensin-converting enzyme I/D polymorphism: relationships with gender, hypertension and impaired glucose metabolism

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Background The presence of the deletion allele of the angiotensin-converting enzyme (ACE) I/D polymorphism is associated with an excess risk of vascular disease and diabetic nephropathy.

Objective To examine the importance of this polymorphism as a determinant of hypertension and impaired glucose metabolism in a population-based study of three ethnic groups and assess the potential modifying effect of gender.

Design Population-based cross-sectional study in South London. The population-based sample of 1577 men and women, age 40–59 years, was obtained from stratified random sampling of general practice lists where 25% of the residents were born outside the UK. The ACE I/D polymorphism was determined for 1366 individuals (86.6%): 462 whites, 462 of African descent and 442 of South Asian origin.

Results The genotype frequency within each ethnic group was in Hardy–Weinberg equilibrium. The frequencies were similar in whites and those of African descent (II, ID, DD: 18.4%, 49.6%, 32.0% for whites and 18.4%, 50.5%, 30.9% for those of African descent), but there was a much higher frequency of the II genotype in those of South Asian origin (39.8%, 41.8%, 18.3%; $\chi^2 = 77.6$; $P < 0.0001$). There was no association between the I/D polymorphism and impaired glucose metabolism in any ethnic group. There were also no significant associations between the I/D polymorphism and hypertension in whites and in those of South Asian

origin. This contrasts with a highly significant association between the D allele and hypertension in women of African descent (OR = 2.54; 95% CI 1.38–4.65; $P = 0.003$) but not in men of African descent (0.79; 0.36–1.72) (test for differences between sexes $P = 0.023$).

Conclusions These observations provide estimates of the frequency distribution of the ACE I/D polymorphism in whites, in people of African descent and in people of South Asian origin. Moreover, these results highlight the potential importance of gender-dependent interactions between genetic background and expression of hypertensive phenotype. *J Hypertens* 1999, 17:657–664 © Lippincott Williams & Wilkins.

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Introduction

Hypertension and diabetes are important risk factors for cardiovascular and renal disease [1,2]. A positive association of the angiotensin-converting enzyme (ACE) I/D polymorphism has been observed in some but not every case—control studies of hypertension in white populations [3,4]. However, the influence of ethnic background on the link between hypertension and this polymorphism still remains unresolved. Some studies suggest a higher frequency of the DD genotype in Afro-Caribbeans with essential hypertension [5,6], although this was not confirmed in Jamaicans [7]

or in Caribbeans from St Vincent and the Grenadines [8].

There is also controversy on the potential role of the I/D polymorphism in the development of non-insulin-dependent diabetes mellitus (NIDDM) and impaired glucose metabolism in relation to ethnic origin [9]. While the presence of the D allele seems to be associated with increased risk of myocardial infarction, atherosclerotic and renal microvascular complications in those with diabetes [10], two studies have demonstrated an association between the I allele – rather than

the D allele – and reduced insulin sensitivity [11,12]. In this case, ethnic origin is of particular significance given the higher prevalence of impaired glucose metabolism in those of African or of South Asian origin [13].

Moreover, it is also apparent that, despite earlier studies [14], gender may be an important factor as recent studies have demonstrated an association between the D allele and hypertension in white American men [15] as well as Japanese men [16] but not in women.

The majority of studies examining the associations of the I/D polymorphism with hypertension and diabetes have been case–control studies drawn from clinical settings [3,9]. These are open to important selection biases which may, in part, explain the inconsistency of the findings in the literature. The objectives of the present study therefore were: (a) to compare the frequency distribution of the ACE I/D polymorphism in three population-based samples of middle-aged men and women of three ethnic groups (whites, people of African descent and people of South Asian origin) co-resident in a geographically defined area of South London; (b) to examine the association of the I/D polymorphism with hypertension and with impaired glucose metabolism within each of these ethnic groups; and (c) to study the potential modifying effect of gender.

Methods

Population sample

The population sample was obtained as previously described [17,18]. In brief, a random sample of 1577 men and women aged 40–59 years was obtained from age- and sex-registers of general practitioners in a defined area of South London. They belong to the three main ethnic groups living in the area, namely whites, people of African descent (Caribbeans and West Africans) and people of South Asian Indian origin. Fieldwork was undertaken from March 1994 to July 1996. Ethnic group was recorded at the time of interview, based on the answers to a combination of questions including place and country of birth, language, religion, history of migration and parental country of birth [18]. All participants of ethnic minority groups were first-generation immigrants. The ACE I/D polymorphism was determined on 1366 individuals (86.6%). The study protocol was approved by the local ethics committee. All participants gave their informed consent to participate.

Procedures

Participants attended a dedicated screening unit at St George's Hospital between 0800 and 1200 h, 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, blood pressure and glycosuria measurements were performed as described elsewhere [17,18]. Fasting venous blood was taken in the seated position without stasis. Participants not known to be diabetic and without glycosuria (defined as urinary glucose > 5.5 mmol/l) underwent a standard oral glucose tolerance test [17,18] and venous samples were taken 2 h later. A questionnaire was administered which included personal medical history and drug treatment. As the proportion of participants on anti-hypertensive medication was high and varied significantly by ethnic group [17,18], any further analysis was carried out by categorical variables rather than by blood pressure levels. Hypertension was defined according to the ISH/WHO classification [17,18]. Impaired glucose metabolism (IGM) was defined as the combination of diabetes (known diabetics, those with positive glycosuria or with fasting glucose ≥ 7.8 mmol/l or with 2 h post-load glucose ≥ 11.1 mmol/l) and impaired glucose tolerance by WHO criteria [19].

DNA isolation and ACE I/D genotyping

Whole blood (10 ml) taken from a subcutaneous vein in the forearm was centrifuged for 10 min at 1200 *g* and 4°C, after which the plasma was removed and the remaining buffy coat and erythrocytes stored at –40°C before DNA extraction. Genomic DNA was extracted according to the BACC 2 Nucleon Biosciences protocol (Nucleon Biosciences, Coatbridge, Lanarkshire, UK). After extraction as detailed within the manufacturer's instructions, the DNA was precipitated from the de-canted aqueous phase by the addition of 2 vol ice-cold ethanol. The pelleted DNA was washed with 2 ml 70% ethanol and after air drying resuspended in TE buffer (pH 7.8). The amount of DNA recovered was quantified on a GeneQuant2 spectrophotometer (Pharmacia Biotech). Average yield and purity were 300 µg and a 260/280 ratio of 1.8, respectively.

PCR detection of the ACE I/D polymorphism

The ACE I/D polymorphism was identified using a polymerase chain reaction (PCR)-based method as described previously [20]. Due to the possibility of mistyping of heterozygous DNA by preferential amplification of the deletion allele over the insertion allele in heterozygous samples [21] with the previously described method, 108 of 372 samples (39%) amplified as deletion homozygous using the previous method were re-analysed using the triple primer method [21,22]. All gels were scored by two independent investigators who were not aware of the sample source. Using this method, two of the 108 samples were discordant. This translates into a relatively small rate of mistyping (1.8 %); in view of this – and as there is no apparent reason for preferential mistyping according to ethnic origin or disease status – the results using the first method of genotyping were accepted for analysis.

Statistical analysis

The calculation of allele frequency to test for Hardy–Weinberg equilibrium for each of the three ethnic groups was carried out using standard methods [23]; χ^2 tests were used to compare expected against observed frequencies. Associations between hypertension or IGM and ACE I/D genotype were tested using χ^2 tests. Frequency distributions are expressed as percentages with corresponding approximate confidence intervals [24]. Multiple logistic regression was used to test the effect of the ACE genotype on the likelihood of hypertension or IGM while controlling for confounding factors. The multivariate analysis also provided a means to examine the genetic model for any association between the ACE I/D polymorphism and hypertension or NIDDM. Accordingly, the association between the disease and the ACE genotype was tested by calculating odds ratios under three assumptions [25]. These were: additive, with scores 0, 1, 2 for II, ID and DD respectively; dominant, with scores of 0 for II, and 1 for DI and DD combined; and recessive, with scores of 0 for II and ID combined and 1 for DD. Where appropriate, one-way or two-way analysis of variance was used for group mean comparisons. Logarithmic transformation was used for serum glucose. Group values are given as means \pm SD. All statistical tests were carried out using the Statistical Package for the Social Sciences [26].

Results

Demographic characteristics and prevalence of hypertension and IGM according to ethnic origin

People of African descent were slightly older, heavier and had a higher BMI than the whites (Table 1). The prevalence of hypertension was 2.7-fold higher in those of African descent and 1.7-fold higher in people of South Asian origin compared with whites; by contrast, the prevalence of IGM was 2.7-fold higher in people of

South Asian origin and twofold higher in those of African descent compared with whites (Table 1). Consistent with the prevalence data, blood pressures and glucose levels also showed significant variations by ethnic group (Table 1).

Frequency distribution of ACE I/D polymorphism according to demographic characteristics and ethnic origin

The genotype frequency within each ethnic group was in Hardy–Weinberg equilibrium as the calculated genotype frequencies were not significantly different from those observed. In the group of African descent, 38% were born in West Africa. As there were no significant differences in the I/D genotype frequencies between West Africans (II, ID, DD: 18.1%, 49.6%, 32.2 %) and Caribbeans (20.6%, 53.7%, 25.7 %), these were pooled into one group of people of African descent in subsequent analyses. There were no statistically significant differences in either genotype or allele frequency between whites and people of African descent; by contrast, in those of South Asian origin there was a lower frequency of the D allele and a higher frequency of the I allele (Fig. 1).

Frequency distribution of ACE I/D polymorphism according to gender and hypertension

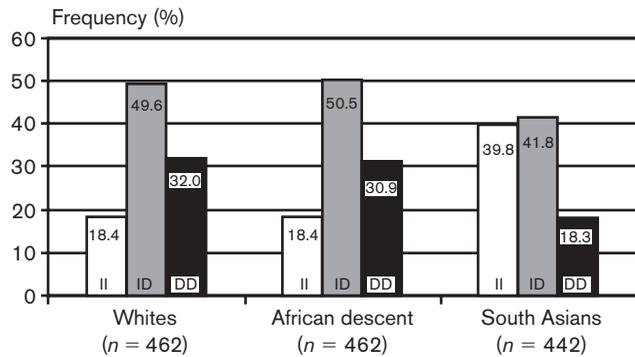
In whites or in people of South Asian origin, there was no significant association between ACE I/D genotype and the prevalence of hypertension within each genotype (Table 2). When the genotype distributions were analysed separately for men and women, in the South Asians there were no differences between the normotensive and the hypertensive group. In the whites there was evidence of a difference between men and women with a higher frequency of the DD genotype in the hypertensive men but not women (27.3 versus 11.8%). However, the significant association found for the white males in the univariate analysis did not persist in the

Table 1 Demographic characteristics, blood pressures and serum glucose in whites, people of African descent and people of South Asian origin

	Whites	African descent	South Asian origin
Number (% women)	462 (55%)	462 (60%)	442 (48%)
Age (years)	49.9 (5.6)	51.2 (5.8)	49.0 (5.8)
Weight (kg)	72.6 (14.1)	77.2 (12.7)	68.7 (12.2)
BMI (kg/m ²)	25.9 (4.6)	28.2 (4.5)	25.9 (4.4)
Systolic BP (mmHg)*	125 (18)	134 (20)	128 (19)
Diastolic BP (mmHg)*	79 (10)	86 (10)	82 (11)
Fasting glucose (mmol/l)	5.06 (0.88)	5.48 (1.98)	5.69 (1.97)
2 h post-load glucose (mmol/l) [†]	5.64 (2.06)	6.08 (2.28)	6.70 (2.66)
Prevalence of			
Hypertension (%)	15.1 (6.6–23.4)	41.5 (34.0–48.0)	25.5 (17.0–33.0)
Impaired glucose metabolism (%)	13.5 (10.0–17.0)	26.7 (22.5–31.5)	36.8 (32.1–41.9)

Values are means (SD) or % (95% CI) as appropriate. BMI, body mass index; BP, blood pressure. *Blood pressure include those of participants on pharmacological treatment. All differences between ethnic groups were significant at $P < 0.0001$ by analysis of variance. [†]Number of subjects: 359 whites; 323 of African descent and 318 of South Asian origin.

Fig. 1



Frequency distributions of angiotensin-converting enzyme I/D polymorphism by ethnic group. The frequency in South Asians was significantly different from the other groups ($\chi^2 = 77.6$; $P < 0.001$). Allele frequencies were also significantly different in South Asians as compared to other groups: I, 0.61 (0.58–0.64) versus 0.44 (0.41–0.47) versus 0.43 (0.40–0.46); D, 0.39 (0.36–0.42) versus 0.56 (0.53–0.59) versus 0.57 (0.54–0.60) in South Asians, people of African descent and whites, respectively ($\chi^2 = 71.6$; $P < 0.001$).

multivariate analysis after adjusting for age, BMI and impaired glucose metabolism. The odds ratios for the white men were 1.28 (0.67–2.45), 0.82 (0.30–2.27) and 2.11 (0.81–5.48) respectively for the additive, dominant and recessive models ($P > 0.1$ in each case).

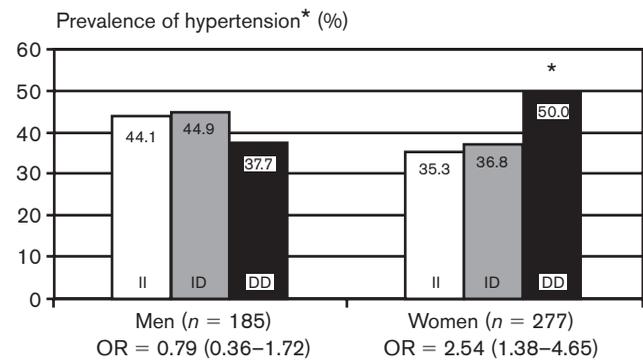
In contrast, in those of African descent there was a tendency for a higher prevalence of hypertension within those of the DD genotype compared with ID or II (45.4 versus 40.1 and 38.8%, respectively), although this did not reach statistical significance. Hypertensive women but not hypertensive men displayed a higher frequency of the DD genotype (50.0 versus 37.7%), but this did not reach statistical significance in the univariate analysis ($P = 0.095$). However, the association between hypertension and the D allele in women but not in men was confirmed by the multivariate analysis. In particular, under the recessive model, although there was no significant association ($P = 0.55$) between ACE

genotype and hypertension in men (OR = 0.79 (0.36–1.72)), there was a highly significant association ($P = 0.003$) between the D allele and hypertension in the women (OR = 2.54 (1.38–4.65)) (interaction $P = 0.023$) (Fig. 2). This significant association between the D allele and hypertension in women of African descent was also confirmed under the additive model for the D allele ($P = 0.007$) with an odds ratio of 1.78 (1.17–2.71).

Frequency distribution of ACE I/D polymorphism according to IGM

In all three ethnic groups, using univariate analysis, there were no significant associations between ACE I/D genotype and IGM (Table 2). The results of the multivariate analyses for the association between the ACE I/D polymorphism and IGM also showed that there was no statistically significant association between ACE genotype and IGM in any of the three ethnic groups (data not shown).

Fig. 2



Angiotensin-converting enzyme I/D genotype distribution according to gender and hypertension in people of African descent. OR, odds ratio (95% confidence intervals). Hypertension is defined as systolic blood pressure ≥ 160 and/or diastolic blood pressure ≥ 95 mmHg or on drug therapy. * $P = 0.023$ as interaction term.

Table 2 Prevalence of hypertension and impaired glucose metabolism according to angiotensin-converting enzyme I/D genotype by ethnic group

	Whites	African descent	South Asians
Hypertension (%)			
II	21.2 (18/85)	38.8 (33/85)	25.0 (44/176)
ID	11.3 (26/229)	40.1 (94/234)	27.0 (50/185)
DD	17.6 (26/148)	45.4 (65/143)	23.4 (19/81)
P value	0.06	0.51	0.81
Impaired glucose metabolism (%)			
II	8.8 (6/68)	24.2 (16/66)	36.5 (54/148)
ID	15.4 (30/194)	24.5 (46/188)	35.5 (55/155)
DD	13.1 (14/107)	31.6 (38/120)	40.3 (29/72)
P value	0.38	0.33	0.78

Discussion

The present study investigated, for the first time, ethnic variations in the frequency of the ACE I/D polymorphism in a well-defined, multi-ethnic population in Britain, and the influence of such polymorphism on hypertension and on IGM in different ethnic groups in London. The study has several novel aspects in addressing genetic variations according to ethnic origin. The study is population-based, with the groups having been studied within the same geographical area, thereby mitigating the potential effects of differences in environmental background, and it examines first-generation immigrants of ethnic minority groups with both parents born in the country of origin and belonging to the same ethnic background, thus markedly reducing the potential impact arising from an unknown degree of admixture.

Ethnic differences in the ACE I/D polymorphism

Previous work in white populations [14] has shown a wide range of estimated frequencies from about 22 to 37% for the DD genotype, compatible with the 32% in the present study. Earlier studies [5,6,27–29] comparing the distribution of the I/D polymorphism between whites and people of African origin suggested a higher frequency of the D allele in populations of African origin (Caribbeans, Nigerians and American blacks). Our results show no difference in the distribution of the ACE I/D polymorphism between whites and people of African ancestry. The higher frequency of the I allele in South Asians is consistent with that found in Chinese [30–33], in Japanese [34–37] and in people from the Indian subcontinent [38]. Little is known of the selective mechanisms leading to such dispersion of the I/D polymorphism with the spread of humans throughout the world. These differences, however, are not only intriguing from the biological point of view, but are also of clinical interest given the known differences in coronary heart disease and diabetes amongst whites, people of African origin and South Asian Indians [13,39]. Whilst the prevalence of diabetes and impaired glucose metabolism are higher in those of African descent and of South Asian origin, coronary heart disease is raised in South Asians but low in people of African origin whether living in Africa, the Caribbean or in Europe [13]. This contrast raises the question of possible genetic susceptibility.

ACE I/D polymorphism, gender and hypertension

The association between the DD genotype and increased levels of plasma and tissue ACE [40,41] provides a theoretical mechanism whereby the I/D polymorphism could be of potential importance in the control of blood pressure. However, in the whites and in the South Asians, there were no differences in the frequency distributions of the I/D polymorphism be-

tween the hypertensives and normotensives even when controlling for potential confounders. This lack of association between the I/D polymorphism and hypertension is in agreement with the majority of previous investigations in whites [3]. Interestingly, there has been one report of a higher frequency of the I allele in hypertensive people of European descent [42]. Subsequently, the same group suggested that this may have been due to increased mortality in the hypertensives of DD genotype [43]. If this were the case, we would have expected those of the II genotype to be older. Our study does not support this view as there was no relationship between I/D genotype and age. Another explanation relates to differences in genetic background. Our participants were not selected according to family history of hypertension, and this contrasts with the stringent criteria used in the selection of subjects with respect to parental phenotype in the previous work. A more heterogeneous family background will dilute the genetic component and emphasize the importance of environmental factors. However, linkage studies in humans have also failed to find an association with blood pressure [44]. Interestingly, the observation of a significant association in white men (although no longer significant when adjusted for confounders) is consistent and compatible with the Framingham study [15], also in whites, which showed a linkage between the ACE locus and hypertension in men but not in women. Whilst the association was attenuated when adjusted for potential confounding factors [15], such results raise the intriguing concept of gender-dependent interactions between the ACE gene and blood pressure.

There have been conflicting reports on the association between the I/D polymorphism and hypertension in other ethnic groups. Despite a series of negative studies from Japan [34,36,37], Morise *et al.* [35] found a higher frequency of the DD genotype in Japanese patients with hypertension compared with normotensives; more recently this has been confirmed in a large population study in Japanese men but not women [16]. Investigations in Chinese people [30–33] reported no association between the I/D polymorphism and high blood pressure; although in one study [30] the frequency of the DD genotype was higher in the hypertensive group compared with the normotensive group (23 versus 13%, respectively), this did not reach statistical significance. To our knowledge, there has only been one report of the I/D polymorphism in people from the Indian subcontinent claiming a significant association with hypertension [38]. This particular investigation examined few people with hypertension belonging to an agricultural, non-smoking, vegetarian Sikh cast. The study did not consider potential confounders, was not population-based and was clearly open to potential selection bias.

Studies of the association between the ACE I/D and hypertension in blacks have been equivocal. Despite two clinic-based investigations [5,6] that reported a positive association between the D allele and hypertension, no associations were found by others [7,8,28,29]. In particular, no association was found in a population-based study of African-Americans [7]. These studies highlight the potential problems associated with clinic-based protocols where cases and controls may not be representative of well-defined populations.

In our study in people of African descent there was a trend for an association between the D allele and hypertension in that, in the multivariate analysis, under the assumption of a recessive model, the odds ratio of 1.65 (1.04–2.64) was statistically significant ($P = 0.035$). However, a more novel finding is the observation of a gender-dependent association between hypertension and the D allele in this ethnic group. Although a gender-dependent association has been reported in white as well as Japanese men, by contrast, in those of African descent, there was a significant association between the D allele and hypertension in women but not in men. Although we cannot entirely exclude the possibility of a type I error, a similar trend was also found under the additive model. The mechanism whereby the presence of the D allele may contribute to the expression of hypertension in women but not in men remains to be resolved as the intronic mutation of the ACE I/D polymorphism in itself is unlikely to be a disease-causing mutation. Despite these limitations, however, these results are of particular interest given the differences in the severity of vascular disease between men and women of African origin [45].

ACE I/D polymorphism and IGM

While differences in ACE activity, if reflected in differences in local production of angiotensin II, could influence glucose metabolism and insulin sensitivity [46], in the present study we found no association between the ACE I/D polymorphism and IGM in all three ethnic groups. Admittedly, the power to detect any differences in the whites was relatively small given the low prevalence of IGM in this ethnic group. Nevertheless, these results are in agreement with previous work in white patients with NIDDM compared to those without NIDDM [9]. However, the major focus of our study was to investigate those of African descent or of South Asian origin, who have a rate of IGM about two- to threefold higher than whites [13,17]. IGM was found in 27% of those of African descent and in 37% of those of South Asian origin. However, we did not find any significant association between the ACE I/D polymorphism and IGM in either of these ethnic groups. Similarly, no significant association was found between the I/D polymorphism and IGM in Chinese people

[31]. By contrast, three out of five case-control studies in Japanese people reported a significant association [9].

We were not able to measure insulin sensitivity as the required methodology is not conducive to population-based studies. However, the results obtained using the oral glucose tolerance test are in striking contrast with those obtained using measures of insulin sensitivity which provide evidence for the I allele rather than the D allele being associated with insulin resistance [11,12,47]. Whether these differences are due to sampling bias or confounding factors (e.g. differences in body mass index) or reflect variability in the pathophysiology of glucose metabolism according to ACE I/D genotype remains to be confirmed. Nonetheless, the presence of the D allele in both diabetic and non-diabetic participants has a substantial deleterious effect on the progression of renal dysfunction especially in those with glomerular disease [48], and, as insulin resistance is a potentially important determinant of ischaemic heart disease in NIDDM [49], this aspect clearly requires further investigation. It seems unlikely, however, that any link between the ACE I/D polymorphism and ischaemic heart disease operates through an effect on the intermediate phenotype of IGM.

Conclusions

The study demonstrates a similar frequency of the ACE I/D polymorphism in whites and in people of African descent; by contrast, a higher frequency of the I allele was found in those of South Asian origin. In all three ethnic groups, there was no association between the I/D polymorphism and IGM. The I/D polymorphism was not associated with hypertension in whites or in South Asians. However, the significant association between the D allele and hypertension in women but not in men of African descent suggests the possibility of gender-dependent interactions between genetic background and the development of hypertension. This aspect warrants further investigation given that gender-dependent associations with hypertension have also been observed for other candidate genes of the renin-angiotensin system [50,51].

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