Aldosterone synthase gene (*CYP11B2*) C-344T polymorphism, plasma aldosterone, renin activity and blood pressure in a multi-ethnic population

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Background **The aldosterone synthase gene (*CYP1B2*) locus is a candidate region involved in the development of hypertension.**

Objective **To study the relationship between the C-344T *CYP1B2* polymorphism, plasma aldosterone, renin activity and blood pressure in a multi-ethnic population.**

Design **Population-based, cross-sectional study of 1313 middle-aged men and women (456 white, 441 of African origin and 416 South Asian). Anthropometry, blood pressure, biochemistry, questionnaire data and timed urine collections were taken with standardized techniques. All were genotyped for the C-344T *CYP11B2* polymorphism.**

Results **The frequency of the C allele was significantly lower in people of African origin (0.21) than in white (0.46) and South Asian (0.43) ($P < 0.001$). After adjustment for age, sex and ethnicity the TT genotype was associated with 14% higher plasma aldosterone levels, 3.7 mmHg higher systolic and 2.1 mmHg higher diastolic blood pressure than CC (P for linear trend < 0.05). No significant interactions with age, sex, ethnicity, body mass index (BMI) and fractional excretion of sodium were found in the associations between genotype and both blood pressure and aldosterone levels. In a sub-sample of participants in which plasma renin activity was measured ($n = 457$), a significant excess of T alleles was found in those with a raised ($\geqslant 750$) aldosterone-to-renin ratio (ARR).**

Conclusion **In this multi-ethnic population, the C-344T CYP1B2 polymorphism is associated with blood pressure, plasma aldosterone levels and ARR. Although significant differences in allele frequencies were found between groups, ethnicity does not explain the results. *J Hypertens* 22:1895–1901 © 2004 Lippincott Williams & Wilkins.**

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Keywords: C-344T aldosterone synthase polymorphism, ethnicity, aldosterone, renin, blood pressure

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Introduction

Alongside monogenic forms of hypertension, a number of relatively common genetic variants appear to be associated with higher blood pressure and increased susceptibility to hypertension. The renin–angiotensin–aldosterone system (RAAS) and other factors that influence the renal sodium handling, through the regulation of the secretion and action of aldosterone, are strong contributors to the development of hypertension [1]. The aldosterone synthase gene, *CYP11B2,* encodes for a cytochrome P450 enzyme, involved in the terminal steps of aldosterone synthesis in the zona glomerulosa cells of human adrenal glands and its expression is regulated by angiotensin II and potassium [2]. The candidacy for this gene is based on its pathogenic role in the syndrome of glucocorticoid-remediable aldosteronism [3]. Several common polymorphisms have been described in the *CYP11B2* [4–7]. The C-344T polymorphism, which is located at a putative binding site for the steroidogenic transcription factor (SF-1), has been associated with hypertension [5,8–10] and with other hypertensive intermediate phenotypes such as plasma aldosterone [11], urinary aldosterone excretion rate [10] and aldosterone–to–renin ratio (ARR) [6–7]. Although some studies have not confirmed these associations [12,13], this locus may be important in blood

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pressure and cardiovascular regulation [14]. Several factors such as gender [15] ethnicity [16] and age [11] could be involved in the phenotypic expression of this polymorphism.

The present study assesses the frequency of C-344T *CYP11B2* polymorphism in three ethnic groups (white, people of African origin and South Asian), living in the same environment (South London) and undergoing a rigorously standardized protocol; it examines whether this polymorphism is associated with blood pressure and aldosterone-related phenotypes and whether the association is modified by known demographic, anthropometric and environmental factors.

Subjects and methods

Population sampling

The study methodology as well as the general characteristics of the population sample are reported in great detail elsewhere [17,18]. In brief, men and women aged 40–59 years were recruited from the lists of general practices in South London between March 1994 and July 1996. Ethnic group was recorded at the time of interview based on the answers to a combination of questions including place of birth, language, religion, history of migration and parental country of birth. The final sample size was 1577. Of those, 1379 (87%) were genotyped for C-344T *CYP11B2* polymorphism. A complete data set was available in 1313 (83%) subjects (456 white, 441 of African origin and 416 South Asian). Urine collections were also available in 1131 (72%) of them (409, 376 and 346 for each ethnic group, respectively). Participants from ethnic minority groups were all first generation immigrants. The general characteristics of those included in the analysis did not differ from those who were excluded (Appendix 1). The study was approved by the Local Ethics Committee. All participants gave their informed consent to participate.

Methods

Participants were seen between 0800 and 1200 h after an overnight fast. They received written instruction to void their bladder in the morning, to record the time and to drink one–two glasses of tap water before attending the screening. They were asked to refrain from smoking and from taking vigorous exercise for at least 1 h before the visit and to bring all medications with them for checking. The examination included anthropometry, blood pressure, a fasting timed urine collection and a detailed questionnaire that included demographic and socio-economic information such as place of birth, language, religion, history of migration, parental place and country of birth, family history, marital status, social class and education for both screened and spouse/partner (when indicated), housing. It also included personal medical history and drug therapy, current and past smoking, leisure-time physical activity over the preceding fortnight. Age was recorded at the last birthday. Height, weight, waist and hip girths were measured with standard methods [17,18] and body mass index (BMI) was calculated (kg/m^2). Blood pressure (BP) was measured after the subject had been resting for at least 10 min in the supine position with an automatic ultrasound sphygmomanometer as described elsewhere [17,18].

After the physical measurements participants completed a timed urine collection having fasted from the night before. Urine samples were stored at −20°C until assayed for sodium and creatinine concentrations. Total fractional excretion of sodium (FE Na) was used as a crude indicator of dietary salt intake [19,20].

Fasting venous blood was taken in the seated position without stasis. Serum electrolytes and creatinine were measured as previously described [17,18]. Plasma aldosterone levels were measured in the same laboratory by radioimmunoassay (RIA) [21]. Plasma renin activity was measured by RIA in a sub-sample of 457 participants [22].

C-344T CYP11B2 polymorphism

Genomic DNA was extracted according to the BACC 2 Nucleon Biosciences protocol (Nucleon Biosciences, Coatbridge, Lanarkshire, UK), as previously described [23]. The amount of DNA recovered was quantified on a GeneQuant2 spectrophotometer (Pharmacia Biotech, Milan, Italy). Average yield and purity were 300 μg and a 260/280 ratio of 1.8, respectively.

The genomic region encompassing the biallelic polymorphism (C-344T) of *CYP11B2* was amplified from each DNA sample by the polymerase chain reaction (PCR) in 10 μl reactions containing 0.025 U *Taq* DNA polymerase, 1 × concentration of the buffer supplied, 0.2 mmol/l concentration of each deoxynucleotide triphosphate, and 10 pmol of each primer. PCR conditions were: initial denaturation at 94°C for 2 min; then, 30 cycles at 94°C for 30 s, at 67°C (annealing) for 30 s, at 72°C (extension) for 30 s; final extension at 72°C for 5 min. Subjects were genotyped for the -344 promoter polymorphism using primers CAGGAGGAGACCC CATGTGAC (sense) and CCTCCACCCTGTTCA GCCC (antisense). Restriction fragment length polymorphism analysis (RFLP) was performed by adding 10 U of restriction endonuclease *Hae* III (Gibco BRL) in the appropriate buffer to 5 μl from each reaction (a 537-bp product) and by incubating at 37°C for 2 h. The samples digested then underwent electrophoresis on 2.0% agarose gel with a Gel Electrophoresis Apparatus GNA-200 (Pharmacia Biotech), ethidium bromide stained, and analysed under UV-light [11]. Since the -344T allele lacks an Hae III site (GGCC) present in

the -344C allele, the -344T alleles are detected as fragments of 273 bp and -344C alleles as fragments of 202 bp. A 10% random sample of the study population was double genotyped in a blinded fashion with 100% concordant results.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS-PC; SPSS Inc. Chicago, Illinois, USA). The distributions of plasma aldosterone, plasma renin activity and aldosterone-to-renin ratio (ARR) were normalized by log-transformation, and log-transformed values were used in the analyses. Results are expressed as means or geometric means and 95% confidence intervals (CIs) as indicated. Analysis of co-variance and multiple linear regression analysis were used to allow for confounders using systolic, diastolic blood pressure and aldosterone as dependent variable. Due to significant age and sex differences all statistical analyses were carried out after age and sex adjustment. The genotype effect was tested using an additive model, by entering in the equations the C-344T alleles in the *CYP11B2* (1, 2 and 3, corresponding to CC, TC and TT genotypes) as explanatory factor. The interaction between genotype and known explanatory factors of blood pressure and aldosterone variability was evaluated by adding to the standard model with age, sex, ethnicity and BMI, in turn, the 'age × genotype', 'sex × genotype', 'ethnicity × genotype' or 'body mass × genotype' product terms, respectively. The C/T allele frequency distribution and the prevalence of antihypertensive drug treatment in different ethnic groups were tested by χ^2. Two-sided P value < 0.05 was considered statistically significant.

Results

Descriptive statistics

A total of 1313 participants were included in the main analysis. The overall characteristics of the study population and the differences between ethnic groups, adjusted for age and sex, in participants genotyped for the C-344T *CYP11B2* polymorphism are shown in Table 1. BP and BMI were lower in white participants and plasma aldosterone was lower in people of African origin. Hypertension and its treatment were more common among blacks. In spite of similar collection time, people of African origin displayed lower urinary volume and fractional sodium excretion than the other groups. All these differences between groups were not due to differences in the proportion of people treated for hypertension, hyperlipidemia or diabetes [24].

Genotype and allele frequencies

The distribution of the C/T alleles in each ethnic group was in accordance with the Hardy–Weinberg equilibrium. The frequency of the C allele was significantly lower in people of African origin (0.21) than in white (0.46) and South Asian (0.43) ($P < 0.001$) (Table 2).

Plasma aldosterone and blood pressure according to the C-344T CYP11B2 polymorphism

There was a significant and positive association between the presence of the T allele and plasma aldosterone levels (Fig. 1a). This association was independent of age, sex and ethnic groups. Likewise, the presence of the T allele was associated with higher systolic and diastolic blood pressures (Fig. 1b, c), independently of confounding factors. Homozygotes for the T allele had 41 pmol/l higher plasma aldosterone levels, 3.7 mmHg higher systolic and 2.1 mmHg higher diastolic blood pressure than homozygotes for the C allele. Heterozygotes showed intermediate levels (data not shown). No significant differences were detected in serum electrolytes and BMI (data not shown). A model adjusted for fractional excretion of sodium did not substantially alter this pattern of associations (data not shown). Finally, no significant interactions with age, gender, ethnic group

Table 1 Age and sex adjusted characteristics by ethnic group in participants genotyped for C-344T *CYP11B2* polymorphism

	White ($n = 456$)		African origin ($n = 441$)		South Asian ($n = 416$)		
	Mean	95% CI	Mean	95% CI	Mean	95% CI	*P*
Systolic BP (mmHg)	125	124–127	134	132–135	129	127–131	< 0.001
Diastolic BP (mmHg)	80	79–81	86	85–87	83	82–84	< 0.001
Body mass index (kg/m^2)	25.7	25.3–26.1	28.0	27.6–28.4	26.0	25.5–26.4	< 0.001
Serum sodium (mmol/l)	140	139–140	140	140–140	139	139–139	< 0.001
Serum potassium (mmol/l)	4.26	4.23–4.28	4.15	4.12–4.17	4.26	4.24–4.29	< 0.001
Plasma aldosterone (pmol/l)*	373	355–391	302	287–317	346	329–365	< 0.001
BP treatment (*n* %)	41 (9.0)		141 (32.0)		59 (14.2)		
Timed urine collections†							
Time (min)	150	145–156	157	151–163	154	148–160	0.221
Volume (ml)	295	277–314	246	226–265	277	258–297	0.001
Creatinine clearance (ml/min)	90.9	88.0–93.8	95.8	92.7–98.8	74.9	71.8–78.1	< 0.001
FE Na (%)	0.82	0.77–0.86	0.79	0.74–0.84	0.99	0.95–1.04	< 0.001

*Geometric means; †subgroup in which urine collections were available. BP, blood pressure; CI, confidence interval, FE Na, fractional excretion of sodium.

Table 2 Genotype and allele frequencies of the C-344T *CYP11B2* polymorphism by ethnic group

	White ($n = 456$)	African origin ($n = 441$)	South Asian ($n = 416$)
Genotype (*n* (%))			
T/T	142 (31.1)	274 (62.1)	132 (31.7)
T/C	216 (47.4)	150 (34.0)	205 (49.3)
C/C	98 (21.5)	17 (3.9)	79 (19.0)
Allele			
T	0.55	0.79	0.56
C	0.45	0.21	0.44

$\chi^2 = 121.3$, $P < 0.001$

BMI and fractional excretion of sodium were detected in multivariate models (Table 3).

ARR according to the C-344T CYP11B2 polymorphism

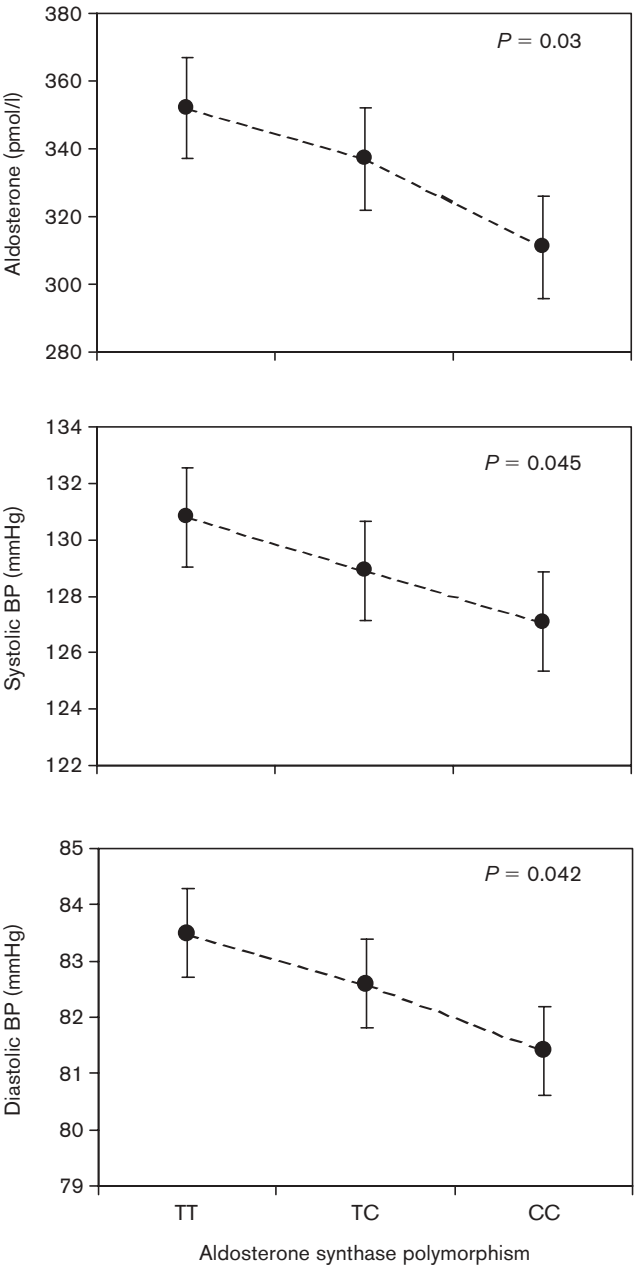
In a sub-sample of 457 individuals in which plasma renin activity (PRA) was measured, age-, sex-, and ethnicity-adjusted ARR was significantly higher in TT as compared with CC (Table 4). Moreover, by using an ARR threshold of 750, a statistically significant excess of the T allele was observed in individuals with high ARR (Table 5).

Discussion

In this multi-ethnic population-based cross sectional study of middle-aged men and women, we observed significant differences in the allele frequency of the C-344T *CYP11B2* polymorphism between ethnic groups. In particular, the allele frequency in whites was similar to that found in previous studies of Caucasian populations [5,8,11], while the frequency of the C allele in people of African origin was similar to that found in a previous study [16] and significantly lower than in white and in South Asian individuals. To our knowledge, this is the first report on the C-344T allele frequency in a South Asian population from the Indian sub-continent. Interestingly, the allele frequency reported in a Japanese population [25] showed an intermediate value between that of white and South Asian on one side and people of African origin on the other, thus confirming the existence of an ethnic and/or geographic variability in the distribution of this allelic variant. The reasons for these differences in allelic distribution in people of different geographical origin are not clear. However, despite the differences in allelic distribution, in the present study ethnicity did not influence the relation between the C-344T *CYP11B2* polymorphism, the renin–angiotensin–aldosterone system and blood pressure.

In the whole population, blood pressure and plasma aldosterone levels were higher in the carriers of the T allele of the C-344T *CYP11B2* polymorphism independently of ethnicity and of other factors possibly affecting these relationships such as sex, age, BMI and fractional excretion of sodium.

Fig. 1



Age, sex and ethnicity adjusted characteristics by aldosterone synthase polymorphism in the whole population ($n = 1313$). *P* for linear trend; BP, blood pressure.

Previous data on the association between the C-344T *CYP11B2* polymorphism and blood pressure or with hypertensive intermediate phenotypes such as plasma aldosterone are discordant. In white populations some case–control studies suggested a positive association of the T allele with hypertension [8,10]. Others did not find any association [12,13], while a recent paper by Kumar *et al.* reported that the C allele was associated with hypertension in Caucasian women [5]. No associa-

Table 3 Interactions between genotype and known explanatory factors of blood pressure and aldosterone variability

Interaction of genotype with:	Dependent variables								
	Systolic BP			Diastolic BP			Log Aldosterone		
	β	95% CI	*P*	β	95% CI	*P*	β	95% CI	*P*
Age	−0.03	−0.27–0.21	0.786	−0.03	−0.16–0.10	0.669	−0.001	−0.007–0.007	0.940
Gender									
Female*	−1.87	−4.67–0.93	0.190	−1.01	−2.55–0.53	0.198	−0.01	−0.09–0.07	0.848
Ethnicity									
South Asian†	−1.09	−4.49–2.31	0.798	0.14	−1.73–2.01	0.689	0.05	−0.05–0.15	0.474
African origin†	−0.91	−4.69–2.87		−0.76	−2.84–1.32		0.06	−0.05–0.17	
BMI	−0.12	−0.43–0.18	0.440	−0.13	−0.30–0.04	0.127	−0.004	−0.014–0.005	0.340
FE Na[c]	1.59	−1.56–4.74	0.324	0.01	−1.73–1.74	0.993	−0.006	−0.09–0.08	0.904

$n = 1313$; *male parameter was set to zero because it was redundant in the analysis. †White parameter was set to zero because it was redundant in the analysis. [c]sub-sample $n = 1131$. The models were carried out after adjustment for age, sex, ethnicity and body mass index (BMI) (and fractional excretion of sodium (FE Na) in the sub-sample). The interactions were evaluated by including in the equation above in turn 'age × genotype', 'gender × genotype', 'ethnicity × genotype' or 'BMI × genotype' product terms, respectively. In the sub-sample 'FE Na × genotype' was used. BP, blood pressure; CI, confidence interval.

Table 4 Plasma aldosterone, plasma renin activity and aldosterone-to-renin ratio by C-344T polymorphism of *CYP11B2*

Variables	C/C ($n = 57$)	T/C ($n = 224$)	T/T ($n = 176$)
Plasma aldosterone (pmol/l)	403 (351–455)	413 (386–440)	417 (383–451)
Plasma renin activity (ng/ml per h)	0.91 (0.76–1.06)	0.94 (0.83–1.05)	0.81 (0.70–0.91)
Aldosterone-to-renin ratio (ARR)†	483 (395–584)	539 (483–602)	652 (572–750)

Results are means or †geometric means (95% confidence interval). †Log transformed values: *P* for linear trend 0.016, adjusted by age, sex and ethnicity.

Table 5 Genotype distribution by ARR

	ARR ⩾ 750 (*n* (%))	ARR < 750 (*n* (%))
C/C ($n = 57$)	13 (22.8)	44 (77.2)
T/C ($n = 224$)	68 (30.4)	156 (69.6)
T/T ($n = 176$)	69 (39.2)	107 (60.8)

χ^2 for linear association 6.4, $P = 0.011$. ARR, aldosterone-to-renin ratio.

tion between the T allele and hypertension has also been reported in some studies in Japanese [26,27] and in one of them hypertension was associated with the C allele [9]. Similar inconsistencies have characterised the reports linking this polymorphism to plasma aldosterone levels [10,13,28,29]. Several reasons could account for these contrasting results. A different genetic background, differences in study design (case–control or cross-sectional designs), differences in selection criteria, such as a different proportion of individuals with low renin hypertension [16] or with different age [11]. In particular, in a white male population, an increase in diastolic and systolic blood pressure and a decrease in aldosterone plasma levels were observed in T carriers across quintile of age, but not in CC homozygotes. These data support the hypothesis that the relationship between this gene variant and blood pressure may become more apparent with increasing age [11]. In the present study, the range of age was very narrow (mean 50.1 years; 95% CI 49.7–50.4) and this could explain why we were unable to detect any interaction with age.

Although recent studies did not support a direct influence of the C-344T variant on the promoter activity of *CYP11B2* [30], the binding of the steroidogenic transcription factor (SF-1) to this region may down-regulate the activity of the promoter by making SF-1 less available to functionally active promoter sites [2]. Moreover, *in vitro* studies showed that C allele binds SF-1 four times more than it does the T allele [31], thus suggesting a modulating effect of this variant on the transcription of the enzyme and, in turn, on the aldosterone secretion. Two recent studies in hypertensive patients showed that a significant excess of the *CYP11B2* T allele was found in patients with relatively higher aldosterone production for the renin level as measured by ARR [6,7]. Although in the present study ARR was available only in about one-third of the whole sample, our results are consistent with previous reports [6,7] and support the view that carriers of the T variant may show an inappropriately high secretion of aldosterone.

Limitations of the study

Antihypertensive treatment was a possible confounding factor. However, adjustments for this variable did not affect the associations described (Appendix 2). We also

adjusted for fractional excretion of sodium, a crude indicator of dietary sodium intake. Although the value of this measure as a precise indicator of dietary intake is limited, nevertheless it is adequate for group comparisons. The influence of this variable as a potential confounder has been rarely considered [11]. In our study, ARR was measured only on a sub-sample. However, the internal consistency of the results across several outcomes and with the results of previous studies conducted in clinical settings [6,7] reduce the likelihood that our results were due to chance alone. According to previous studies, the inclusion of individuals on antihypertensive drug treatment ($n = 85$, 18.6% of the sub-sample) does not appear to influence the interpretation of ARR [6,32–35].

Finally, these results can only be generalized to comparable middle age populations and more studies are needed.

In conclusion, blood pressure, aldosterone plasma levels and ARR were higher in T carriers of the C-344T *CYP11B2* polymorphism. These relations were independent of ethnic origin, gender, age, BMI and fractional excretion of sodium. These results support a potential role for this variant in mechanisms affecting blood pressure regulation and suggest that inappropriate aldosterone secretion may be the link between the *CYP11B2* locus and high blood pressure [14]. Since association studies cannot provide indications of cause–effect relationships, functional genomic approaches are needed to better understand the implications of these findings.

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Appendix 1

Age and sex adjusted characteristics by ethnic group in participants in whom the polymorphism was not determined

	White ($n = 12$)*		African origin ($n = 21$)*		South Asian ($n = 14$)*		
	Mean	95% CI	Mean	95% CI	Mean	95% CI	*P*
Systolic BP (mmHg)	131	121–141	134	127–142	127	117–137	0.031
Diastolic BP (mmHg)	78	73–83	87	83–91	82	77–86	0.174
BMI (kg/m^2)	27.7	24.6–30.7	27.5	25.2–29.8	26.0	23.1–29.0	0.019
Serum sodium (mmol/l)	138	136–140	141	139–142	139	137–141	0.108
Serum potassium (mmol/l)	4.24	4.06–4.41	4.19	4.05–4.32	4.17	4.00–4.35	0.007
Plasma aldosterone (pmol/l)†	366	260–514	267	207–345	458	330–636	0.149
Timed urine collections							
Time (min)	176	149–203	136	116–156	126	100–151	0.164
Volume (ml)	199	138–262	175	128–221	157	97–216	0.023
Creatinine clearance (ml/min)	90.1	69.9–110.4	95.0	79.7–110.3	84.8	65.2–104.3	0.017
FE Na (%)	0.75	0.52–0.97	0.70	0.53–0.87	0.76	0.54–0.97	0.005

*Participants in whom the polymorphism was not determined and in whom all variables were available for the analysis;
†geometric means. BP, blood pressure; BMI, body mass index; FE Na, fractional excretion of sodium; CI, confidence interval.

Appendix 2

Characteristics by T/C polymorphism of *CYP11B2* gene in the whole population ($n = 1131$) adjusted for age, sex, ethnicity, FE Na and anti-hypertensive treatment

	TT ($n = 471$)		TC ($n = 496$)		CC ($n = 164$)		
	Mean	95% CI	Mean	95% CI	Mean	95% CI	*P*
Systolic BP (mmHg)	134	132–136	133	131–135	130	128–133	0.059
Diastolic BP (mmHg)	85	84–86	84.6	84–86	83	81–85	0.050
BMI (kg/m^2)	26.9	26.5–27.4	27.1	26.7–27.6	27.0	26.2–27.7	0.767
Serum sodium (mmol/l)	139	139–140	139.5	139–140	139	139–140	0.849
Serum potassium (mmol/l)	4.18	4.16–1.21	4.20	4.17–4.22	4.17	4.12–4.21	0.805
Plasma aldosterone (pmol/l)*	376	357–397	360	341–379	340	313–369	0.028

*geometric means; BP, blood pressure; BMI, body mass index; CI, confidence interval.