Interaction between the C(−344)T polymorphism of *CYP11B2* and age in the regulation of blood pressure and plasma aldosterone levels: cross-sectional and longitudinal findings of the Olivetti Prospective Heart Study

Paola Russo[a]*, Alfonso Siani[a]*, Antonella Venezia[a], Roberto Iacone[b], Ornella Russo[b], Gianvincenzo Barba[a], Lanfranco D'Elia[b], Francesco P. Cappuccio [c] and Pasquale Strazzullo[b]

Objective **To study the interaction between the C(−344)T *CYP11B2* polymorphism and known determinants (age, body mass and dietary sodium) of blood pressure and plasma aldosterone.**

Design **Cross-sectional and longitudinal (1980–1995) survey of male workers in southern Italy.**

Setting **Medical centre of the Olivetti factories.**

Participants **In 1995, the C(−344)T polymorphism was characterized in 811 untreated men. A subgroup of 280 participants already seen in 1980 was the object of longitudinal analysis.**

Main outcome measures **Blood pressure, demographic, anthropometric and biochemical variables (serum and urinary electrolytes and plasma aldosterone) and frequency of the C(−344)T polymorphism.**

Results **In the whole population, there was no difference among genotypes for any of the variables examined. However, multiple regression showed a significant interaction between age (but not body mass or sodium intake) and genotype with regard to systolic ($P = 0.03$) and diastolic ($P = 0.02$) pressure variability independently of covariates. Diastolic pressure increased linearly with age in carriers of the T allele (TT, $P < 0.001$ and TC, $P = 0.005$), but not in CC homozygotes ($P = 0.848$). In T carriers – but not in CC homozygotes – blood pressure and serum potassium increased and plasma aldosterone and serum sodium decreased across quintiles of age ($P < 0.001$ for all trends). In the longitudinal study, diastolic pressure increased significantly over time only in T carriers (TC+TT: +2.6 ± 0.6, versus CC: −0.4 ± 1.5 mmHg, $P = 0.04$).**

Conclusion **Inter-individual variation of blood pressure and plasma aldosterone is affected by the interaction of *CYP11B2* C(−344)T polymorphism and ageing, thus supporting a role for this variant in mechanisms affecting blood pressure regulation. *J Hypertens* 20:1785–1792 © 2002 Lippincott Williams & Wilkins.**

Journal of Hypertension 2002, **20**:1785–1792

Keywords: ***CYP11B2*, aldosterone synthase, polymorphism, blood pressure, ageing, aldosterone**

[a]Epidemiology and Prevention, Institute of Food Science and Technology, National Research Council of Italy, Avellino, Italy; [b]Department of Clinical and Experimental Medicine, Unit of Clinical Genetics and Pharmacology, Hypertension and Mineral Metabolism, 'Federico II' University of Naples, Naples, Italy; [c]Department of General Practice and Primary Care, St. George's Hospital Medical School, University of London, London, UK.

Sponsorship: The study was supported in part by a grant from the Italian Ministry of University and Scientific Research and by Modinform S.p.A (Olivetti group).

Correspondence to Alfonso Siani, MD, Institute of Food Science and Technology-CNR Via Roma 52 A/C, 83100 Avellino, Italy.
Tel: +39 0825 299353; fax: +39 0825 781585; e-mail: asiani@isa.av.cnr.it

or to Pasquale Strazzullo, MD, Department of Clinical and Experimental Medicine, 'Federico II' University of Naples, Via S. Pansini 5, 80131, Naples, Italy.
Tel: +39 081 7463686; fax: +39 081 5466152; e-mail: strazzull@unina.it

Received 7 February 2002 **Revised** 22 April 2002
Accepted 27 May 2002

Introduction

The role of genes regulating the components of the renin–angiotensin system and/or the factors involved in aldosterone production as a possible candidate for arterial hypertension has been studied extensively [1,2]. In particular, the candidacy for the *CYP11B2* (aldosterone synthase gene) is based not only on its functional relevance for the synthesis of aldosterone but also on its pathogenic role in the syndrome of glucocorticoid-remediable aldosteronism [3], a rare form of inherited hypertension. Therefore targeted investigations have been conducted to explore the contribution of other variants of this gene to susceptibility for essential hypertension.

Among the frequent polymorphisms described for the *CYP11B2* gene, the C(−344)T polymorphism in its transcriptional regulatory region has been reported to

*P.R. and A.S. contributed equally to the study.

be associated with hypertension [4–7] or with intermediate phenotypic indicators of aldosterone secretion [6–9]. However, the results of these studies have been controversial [10,11] and firm conclusions on the possible role of this variant on blood pressure and other related phenotypic variables have not been reached. Explanations for the inconsistencies are not obvious. Most of the studies carried out so far have been case–control studies involving selected hypertensive patients and their normal counterparts [4–7]. Most of them included patients on antihypertensive medications known to possibly affect both blood pressure and aldosterone levels, thus limiting their phenotypic characterization. Another reason for the inconsistency among various reports is that the phenotypic expression of a given susceptibility genotype may be confounded by the concomitant influence of environmental and personal factors (e.g. age, gender and race) with known and measurable effects on the inter-individual variance of the parameter under investigation. In particular, both blood pressure and aldosterone secretion regulation are influenced by factors such as age [12,13], body mass [14,15] and dietary sodium intake [16,17]. Previous studies suggested that the effects of *CYP11B2* C(−344)T polymorphism on circadian variation of blood pressure [18] and other cardiovascular phenotypes, such as left ventricular mass [11,19] and baroreflex sensitivity [20], could be age-dependent.

The aim of the present study was to investigate the association of the *CYP11B2* C(−344)T polymorphism with blood pressure, plasma aldosterone and other parameters of electrolyte homeostasis and to evaluate the interactions of this polymorphism with known demographic, anthropometric and environmental determinants (age, body mass and dietary sodium intake) of these phenotypic variables in a large sample of untreated workers attending the 1994–95 examination of the Olivetti Prospective Heart Study.

Methods

Population and field procedures

The study was performed at the Olivetti factories in Pozzuoli (Naples) and Marcianise (Caserta) and was part of a longitudinal investigation on the prevalence of cardiovascular risk factors in southern Italy involving the participation of the Olivetti factory male workforce. The methodology of the study has been described in detail elsewhere [21,22]. The study protocol was approved by the local Ethics Committee and participants gave their informed consent to participate.

Between May 1994 and December 1995, 1075 men in the age range 25–75 years were examined. The C(−344)T *CYP11B2* polymorphism was characterized in 998 participants. After the exclusion of 187 men on antihypertensive drug treatment, a complete data set was available for 811 men who were included in the present analysis. A subgroup of the untreated subjects ($n = 280$) had already been seen in 1980 and was the object of the follow-up analysis.

The examinations were performed in the morning, in a quiet and comfortable room within the medical centre of the Pozzuoli and Marcianise factories: the study included a physical examination, anthropometric measurements, a resting 12-lead electrocardiogram, a blood test and a fasting timed urine collection. A fixed-sequence questionnaire was administered, and included demographic information and past medical history. A self-administered semiquantitative food frequency questionnaire was used for the assessment of dietary habits.

Blood pressure and anthropometric measurements

Blood pressure was measured between 08 00 h and 11 00 h, after the subject had been sitting upright for at least 10 min. Systolic and diastolic (phase V) blood pressure measurements were taken three times, 2 min apart, with a random zero sphygmomanometer (Gelman Hawksley Ltd, Sussex, England). The first reading was discarded and the average of the second two readings was recorded for systolic and diastolic blood pressure.

Body weight and height were measured on a standard beam balance scale with an attached ruler. Body weight was measured to the nearest 0.1 kg and height was measured to the nearest centimetre, with subjects wearing only light indoor clothing without shoes. The body mass index was calculated as weight in kilograms divided by the square of their height in metres.

Both anthropometric and blood pressure measurements were performed by trained observers who had attended training sessions for standardization of the procedures. The operator code was recorded in order to check for possible measurement biases.

Fasting urinary collection, blood sampling and biochemical assays

On the morning of the study, after voiding and discarding overnight urine and drinking 400 ml of tap water, participants produced a fasting timed urine collection. The collection time and volume were recorded and a specimen was used for the analysis. At the mid-point of the urine collection and after the blood pressure measurements, a blood sample was obtained by venepuncture, with the subject in the seated position and without stasis, between 08 00 and 11 00 h. Biochemical determinations on serum and urine samples were performed as described below.

Blood specimens were immediately centrifuged and stored at −70°C until analysed. Creatinine was meas-

ured by the picric acid colorimetric method, serum and urinary electrolytes by atomic absorption spectrophotometry. Plasma aldosterone was measured by radioimmunoassay (DRG Instruments, GmbH-Germany) on 673 participants for whom a plasma sample was available. No difference in age, blood pressure and body mass was found between this group and the remaining study participants.

Creatinine clearance was calculated, corrected for body surface area when appropriate, and used as an index of glomerular filtration rate. Urinary sodium and potassium excretion rates on morning timed urine collection were calculated and taken as a surrogate index of dietary intake.

C(−344)T *CYP11B2* gene polymorphism

Genomic DNA was isolated from leucocytes with a non-enzymatic, salting out procedure [23]. Segments of *CYP11B2* were amplified from each DNA sample by the polymerase chain reaction (PCR) in 20 μl reactions containing 0.03 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 5 min; then 35 cycles at 94°C for 1 min, at 67°C (annealing) for 1 min, and at 72°C (extension) for 2 min.

Subjects were genotyped for the −344 promoter polymorphism using primers CAGGAGGAGACCCCAT GTGAC (sense) and CCTCCACCCTGTTCAGCCC (antisense). Restriction fragment length polymorphism analysis (RFLP) was performed by adding 10 U of restriction endonuclease *Hae*III (Gibco-Invitrogen, Carlsbad, California, USA) in the appropriate buffer to 5 μl from each reaction (a 537 bp product) and by incubating at 37°C for 2 h [19]. The samples digested then underwent electrophoresis on 2.5% agarose gel with a Gel Electrophoresis Apparatus GNA-200 (Pharmacia Biotech, Milan, Italy), ethidium bromide stained, and analysed under UV light. Since the (−344)T allele lacks an *Hae*III site (GGCC) present in the (−344)C allele, the (−344)T alleles are detected as fragments of 273 bp and (−344)C alleles as fragments of 202 bp (plus smaller fragments in each case).

A 10% random sample of the study population was double genotyped in a blinded fashion with concordant results.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS-8.0, Chicago, Illinois, USA). As plasma aldosterone and serum electrolyte values did not follow a Gaussian distribution, log-transformation was executed and the log-transformed values were used for the analyses. One-way analysis of variance was used to evaluate differences between group means. Linear regression models were calculated: (1) to assess whether variation in *CYP11B2* genotype made a statistically significant contribution to systolic and diastolic pressure variability allowing for the concomitant effects of known determinants of blood pressure variability such as age, body mass index and dietary sodium intake; (2) to evaluate whether the interaction between genotype and each of the abovementioned factors affected blood pressure variability. The genotype effect was tested by entering in the equations the number of −344T alleles in the *CYP11B2* (0, 1 and 2, corresponding to CC, TC and TT genotypes) as explanatory factor. The interaction between genotype and known explanatory factors of blood pressure variability was evaluated by including in the equation the 'age × genotype', 'body mass × genotype' and 'dietary sodium intake × genotype' product terms, respectively. The associations found in multivariate analysis were reported as multiple regression coefficients (b) and 95% confidence interval (CI). Simple linear correlation and regression analyses were used to test the bivariate associations between different variables in the whole population and among *CYP11B2* genotypes. To analyse the effect of age on selected dependent variables in relation to the C(−344)T polymorphism, the population sample was divided into quintiles of age. The presence of a linear trend in mean values of the variables considered was tested across the quintiles of age in relation to the genetic background. Results are expressed as means and SDs or 95% CI, as indicated. Two-sided P values below 0.05 were considered statistically significant.

Results

C(−344)T *CYP11B2* genotyping

Eight-hundred and eleven participants were included in the analysis [age 51.2 ± 7.4 years, body mass index (BMI) 26.7 ± 2.9 kg/m^2, systolic blood pressure 127.3 ± 15.7 mmHg, diastolic blood pressure 82.7 ± 9.2 mmHg (mean ± SD)]. Genotype frequency did not deviate from the Hardy–Weinberg equilibrium. The CC genotype was present in 182 individuals (22.4%), the TC genotype in 417 individuals (51.4%) and the TT genotype in 212 (26.2%). The frequency of the T allele (0.52) was similar to that described in previous studies on Caucasian populations [4,6,10].

Association of phenotypic variables with C(−344)T *CYP11B2* genotypes

A comparison of selected variables in relation to C(−344)T *CYP11B2* genotypes is shown in Table 1. No significant difference was detected in any of the variables investigated, including dietary sodium and potassium as measured by food frequency question-

Table 1 **Characteristics of the population ($n = 811$) according to *CYP11B2* C(−344)T genotypes**

Variable	TT ($n = 212$)	TC ($n = 417$)	CC ($n = 182$)
Age (years)	51.5 ± 7.5	51.4 ± 7.1	50.3 ± 7.7
BMI (kg/m^2)	26.7 ± 2.9	26.8 ± 2.8	26.5 ± 3.1
Systolic BP (mmHg)	127.5 ± 16.7	127.0 ± 15.1	127.7 ± 15.7
Diastolic BP (mmHg)	82.6 ± 9.6	82.8 ± 8.9	82.4 ± 9.5
Creatinine clearance (ml/min per m^2)	50.7 ± 13.7	49.8 ± 13.5	49.1 ± 12.2
Plasma aldosterone[a] (pmol/l)	277.5 ± 166.9	277.0 ± 167.3	294.9 ± 192.2
Serum Na^+ (mmol/l)	139.3 ± 2.0	139.3 ± 2.1	138.9 ± 2.2
Serum K^+ (mmol/l)	4.8 ± 0.4	4.8 ± 0.4	4.8 ± 0.4
Dietary Na^+ (mg/1000 kcal)	1036.8 ± 252.1	1054.2 ± 261.2	1036.9 ± 254.2
Dietary K^+ (mg/1000 kcal)	1606.5 ± 325.5	1600.4 ± 334.2	1575.9 ± 299.5
U-Na^+ rate (μmol/min) [b]	156.4 ± 85.4	149.2 ± 76.5	142.8 ± 71.5
U-K^+ rate (μmol/min)[c]	57.8 ± 26.7	55.1 ± 28.0	52.6 ± 24.1

Values are means ± SD. BMI, body mass index. [a]Data available for $n = 673$ (CC = 152; TC = 350; TT = 171). [b]Urinary sodium excretion rate. [c]Urinary potassium excretion rate.

naires and urinary excretion rates of sodium and potassium, taken as surrogate measures of intake.

Analysis of the interactions between C(−344)T *CYP11B2* genotypes and determinants of blood pressure variability

The possible effect of C(−344)T *CYP11B2* polymorphism on inter-individual blood pressure variance was explored by multiple regression analysis, allowing for the concomitant effect of known explanatory factors (such as age, body mass index and indicators of dietary sodium intake). As shown in Table 2, panel A, variation in *CYP11B2* genotype did not make a statistically significant contribution to prediction of blood pressure, body mass and age being the sole significant determinants for both systolic and diastolic pressure. However, when the product term 'age × *CYP11B2* genotype' was added in the equation, most of the independent effect of age on BP variability appeared to be explained by the statistically significant interaction between age and genotype (Table 2, panel B). Conversely, blood pressure variability was not significantly affected by the inclusion in the regression equation of the product terms 'body mass × *CYP11B2* genotype' or 'sodium intake × *CYP11B2* genotype' (as measured by food frequency questionnaires or by urinary excretion rate), respectively (equations not shown).

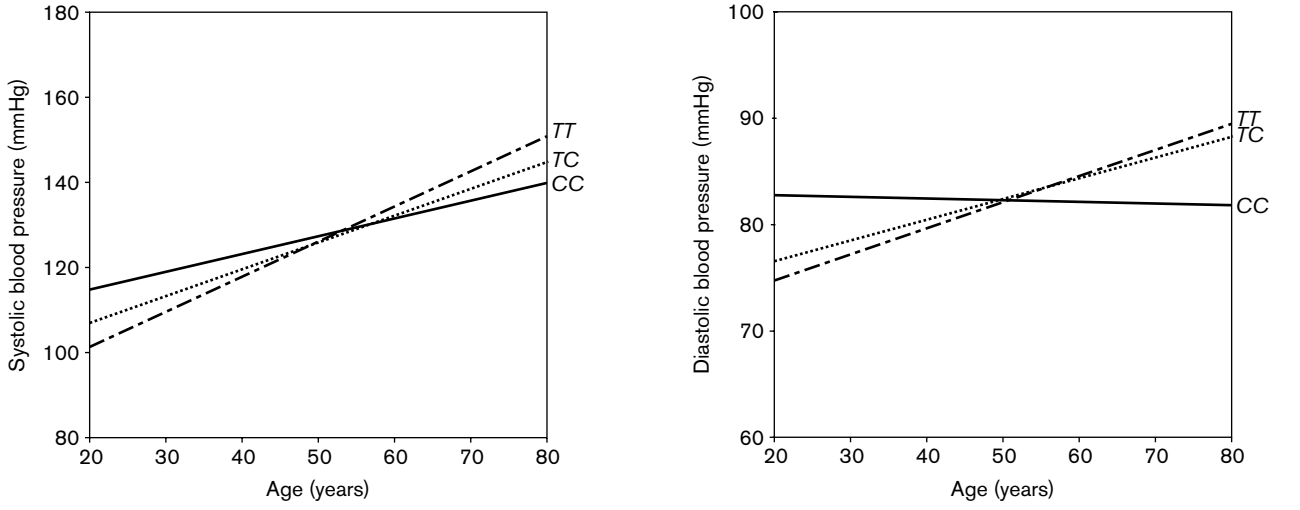
We thus conducted additional analyses to evaluate whether the relationships between blood pressure and age did vary among *CYP11B2* genotypes. As expected, in the whole population both systolic ($r = 0.292$, $P < 0.001$) and diastolic ($r = 0.126$, $P < 0.001$) blood pressure were significantly and positively associated with age. However, separate analyses indicated genotype-dependent different trends for carriers of the T allele and CC homozygotes (Fig. 1). In subjects carrying the T allele (TC and TT genotypes), age was significantly and positively associated with both systolic and diastolic blood pressure; the strength of the association appeared to increase with the number of T allele copies. By contrast, CC homozygotes presented no association between diastolic blood pressure and age,

Table 2 **Stepwise multivariate regression equations, systolic and diastolic blood pressure as dependent variables**

	Systolic blood pressure		Diastolic blood pressure	
Explanatory factor	B (95% CI)	*P* value	B (95% CI)	*P* value
Panel A				
Body mass index (kg/m^2)	0.817 (0.468, 1.166)	< 0.001	0.744 (0.532, 0.955)	< 0.001
Age (years)	0.589 (0.450, 0.728)	< 0.001	0.146 (0.06, 0.231)	0.001
U-Na^+ rate[a] (μmol/min)	0.004 (−0.009, 0.017)	0.533	0.0004 (−0.008, 0.008)	0.999
CYP11B2 genotype[b]	−0.632 (−2.1, 0.8)	0.399	−0.036 (−0.929, 0.856)	0.936
Panel B				
Body mass index (kg/m^2)	0.819 (0.471, 1.167)	< 0.001	0.745 (0.534, 0.956)	< 0.001
Age (years)	0.163 (−0.246, 0.572)	0.434	−0.122 (−0.370, 0.125)	0.332
U-Na^+ rate[a] (μmol/min)	0.004 (−0.009, 0.017)	0.537	0.0003 (−0.008, 0.008)	0.995
CYP11B2 genotype[b]	−11.4 (−21.2, −1.6)	0.023	−6.8 (−12.8, 0.877)	0.025
'Age × *CYP11B2* genotype'	0.212 (0.021, 0.402)	0.03	0.134 (0.02, 0.249)	0.024

Multiple regression coefficients (B) and 95% confidence interval (CI) of B are given for the effects of the factors selected into each model. [a]Urinary sodium excretion rate. [b]−344CC = 0, −344TC = 1, −344TT = 2.

Fig. 1



Genotype-dependent linear regression of blood pressure level on age. Systolic blood pressure (left panel): TT ($n = 212$) $r = 0.364$, $P < 0.001$; TC ($n = 417$) $r = 0.295$, $P < 0.001$; CC (n = 182) $r = 0.204$, $P = 0.006$. Diastolic blood pressure (right panel): TT ($n = 212$) $r = 0.192$, $P = 0.005$; TC ($n = 417$) $r = 0.158$, $P = 0.001$; CC ($n = 182$) $r = -0.014$, $P = 0.848$.

and the association between age and systolic blood pressure was weaker, albeit still statistically significant.

The regression slope of diastolic blood pressure on age was not significantly different from zero in the CC group. Statistically significant differences were found by comparing CC with both TC (mean difference between slopes 0.213 mmHg/year, 95% CI of the difference 0.001–0.427, $P = 0.05$) and TT subjects (mean difference between slopes 0.264 mmHg/year, 95% CI of the difference 0.01–0.513, $P = 0.03$).

Genotype-dependent relationships between phenotypic variables and age

Given the different behaviour observed in T carriers and C homozygotes, the population was divided into two groups: subjects with T allele (TC+TT group) and subjects without (CC group). The sample was thus stratified by quintiles of age, and the presence of a linear trend in mean values of phenotypic variables, such as blood pressure, plasma aldosterone and serum electrolytes, was tested across the quintiles of age in the two groups (Table 3, A–B). In the T carriers, a graded and statistically significant increase in both systolic and diastolic blood pressure was observed across quintiles of age. In this group, a statistically significant age-related trend to a decrease in plasma aldosterone and serum sodium levels and to an increase in serum potassium level was also observed (Table 3, A). In contrast, no similar trends were observed in the CC group, apart from the increase in systolic blood pressure across quintiles of age (Table 3, B).

Follow-up study

A subgroup of the study population ($n = 280$) had been already seen in 1980: 21.9% of these had a CC genotype, 50.8% a TC genotype and 27.3% a TT genotype, a distribution similar to that found in the entire population. At baseline, the two groups (TC+TT and CC) were comparable for age, body mass index and systolic and diastolic blood pressure. Time changes in blood pressure over the 15-year interval between 1980 and the final examination were evaluated according to the two groups previously defined (CC, $n = 63$ versus TC+TT, $n = 217$). Systolic blood pressure changes over the 15-year follow-up were not significantly different between the two groups (TC+TT: $+3.3 \pm 1.0$ mmHg versus CC: $+2.7 \pm 2.3$ mmHg) while an increase in diastolic blood pressure was observed only in the T group (TC+TT: $+2.6 \pm 0.6$ mmHg versus CC: -0.4 ± 1.5 mmHg, $P = 0.04$).

Discussion

The present study is the first population study of the C(−344)T *CYP11B2* polymorphism and blood pressure based on a large sample size and on both cross-sectional and longitudinal observation with long-term follow-up. Most previous studies were case–control studies of selected hypertensive patients and appropriate normotensive controls [4–7]. The analysis carried out on the whole study population did not show a significant association between the C(−344)T *CYP11B2* polymorphism and blood pressure or with intermediate phenotypes such as plasma aldosterone and serum electrolyte levels. Data on the association between the C(−344)T *CYP11B2* polymorphism and blood pressure

Table 3 **Linear trend analysis of selected phenotypic variables across quintiles of age in the TC+TT group (A) and in the CC group (B)**

Variable	Quintile of age					*F* (*P* for trend)
	I (< 47.6 years)	II (47.6–49.4 years)	III (49.4–52.2 years)	IV (52.2–57.2 years)	V (> 57.2 years)	
(A) TC+TT group (*n* = 629)						
Systolic BP (mmHg)	121.3 ± 13.8 (118.9–123.7)	124.5 ± 12.8 (122.2–126.8)	126.4 ± 14.6 (123.9–128.9)	128.8 ± 14.6 (126.2–131.4)	136.7 ± 18.8 (133.1–140.2)	62.9 (< 0.001)
Diastolic BP (mmHg)	80.5 ± 9.4 (78.9–82.2)	81.7 ± 8.7 (80.1–83.3)	83.0 ± 8.7 (81.6–84.5)	83.6 ± 8.5 (82.1–85.0)	85.4 ± 9.8 (83.5–87.2)	19.4 (< 0.001)
Plasma aldosterone (pmol/l)[a]	316.7 ± 171.6 (284.4–349.0)	283.3 ± 159.4 (250.6–315.9)	299.0 ± 210.0 (268.1–330.0)	251.5 ± 136.8 (220.4–282.6)	235.3 ± 133.9 (282.9–267.8)	14.1 (< 0.001)
Serum Na^+ (mmol/l)	139.6 ± 2.1 (139.2–139.9)	139.8 ± 1.7 (139.5–140.1)	139.5 ± 2.1 (139.1–139.9)	139.2 ± 2.3 (138.8–139.6)	138.5 ± 2.0 (138.1–138.9)	19.2 (< 0.001)
Serum K^+ (mmol/l)	4.7 ± 0.4 (4.6–4.8)	4.7 ± 0.3 (4.7–4.8)	4.7 ± 0.4 (4.7–4.8)	4.8 ± 0.3 (4.7–4.8)	4.9 ± 0.4 (4.8–4.9)	11.5 (0.001)
(B) CC group (*n* = 182)						
Systolic BP (mmHg)	125.8 ± 17.8 (120.3–131.3)	125.5 ± 11.5 (122.2–128.9)	124.0 ± 13.8 (118.9–129.1)	128.9 ± 16.0 (122.9–134.9)	136.4 ± 17.3 (129.9–142.9)	8.05 (0.005)
Diastolic BP (mmHg)	83.4 ± 10.8 (80.1–86.7)	83.1 ± 7.1 (81.7–85.2)	78.8 ± 9.0 (75.5–82.2)	82.7 ± 10.1 (78.9–86.5)	83.4 ± 10.6 (79.4–87.3)	0.079 (0.779)
Plasma aldosterone (pmol/l)[b]	302.6 ± 174.7 (237.2–367.9)	280.9 ± 197.4 (216.5–345.4)	242.0 ± 117.2 (163.0–320.9)	335.5 ± 182.0 (263.6–407.3)	306.7 ± 271.0 (233.6–379.8)	0.270 (0.604)
Serum Na^+ (mmol/l)	138.9 ± 2.2 (138.2–139.5)	139.4 ± 1.9 (138.9–140.0)	138.8 ± 3.1 (137.7–139.9)	138.8 ± 2.1 (138.0–139.6)	138.3 ± 1.9 (137.6–139.0)	2.04 (0.54)
Serum K^+ (mmol/l)	4.7 ± 0.4 (4.6–4.8)	4.9 ± 0.4 (4.7–4.9)	4.7 ± 0.3 (4.6–4.8)	4.9 ± 0.4 (4.8–5.1)	4.8 ± 0.4 (4.6–4.9)	0.951 (0.331)

Values are mean ± SD [95% confidence interval (CI)]. [a]Data available for $n = 521$. [b]Data available for $n = 152$.

are discordant, with some case–control studies suggesting a positive association of the −344T allele with hypertension [4,6], some an association with the C allele [5] and others no association [10,11]. Data on the association with indicators of aldosterone secretion are also controversial: while some studies suggested an association between the T allele and higher plasma aldosterone levels [4,8] or higher urinary aldosterone excretion rate [6], respectively, other studies found an association with the C allele [9] or no association at all [11]. Interestingly, however, previous studies suggested that the effects of *CYP11B2* C(−344)T polymorphism on other cardiovascular phenotypes, such as left ventricular mass [11,19] and baroreflex sensitivity [20], might be age-dependent, being more evident in younger rather than in older population samples.

Therefore, we evaluated the interaction of *CYP11B2* C(−344)T polymorphism with age in relation to blood pressure and plasma aldosterone levels in the Olivetti Study population. This analysis showed that the C(−344)T *CYP11B2* genotype affected the relationships of both blood pressure and plasma aldosterone with age to a statistically significant extent. At multiple regression analysis, an independent 'age × genotype' interaction was observed after adjustment for the concomitant effects of other known determinants of blood pressure variability, thus suggesting a genotype-specific impact of age on blood pressure. When the variation of blood pressure with age was estimated by means of separate linear regression analyses, a significant heterogeneity was observed among genotypes. In particular, CC subjects exhibited a different regression line of diastolic pressure on age as compared with T carriers, suggesting a possible regulatory activity of this polymorphism on age-related blood pressure increase. In addition, the trend analysis on cross-sectional data demonstrated that only subjects with the T allele showed a significant increase in diastolic blood pressure across quintiles of age, a finding confirmed by the results of the analysis of the 15-year follow-up study. Systolic blood pressure increased with age in all groups but to a lesser extent in subjects with the CC genotype. In the participants carrying the T allele, a significant and progressive decline of plasma aldosterone levels with age was also observed, and this was associated with a decreasing serum sodium and increasing serum potassium levels.

This pattern is consistent with the previously recognized associations between age, blood pressure and aldosterone secretion. In industrially developed societies, blood pressure levels and prevalence of hypertension tend to increase with age [12]. In our population, the positive correlation between diastolic blood pressure and age is detectable only in the T allele carriers, who represented approximately 75% of

the population. This finding is confirmed by the data of longitudinal analysis on a representative, though smaller, subsample of the cohort. The lower blood pressure increase with age observed in CC subjects is similar to that described for some primitive societies living in a low-sodium environment [24]. Since in our study no differences in salt intake emerged from genotype comparison, these findings suggest that habitual dietary sodium intake may not be the sole factor responsible for the differences in the blood pressure rise with age observed in different populations, a genetic influence also being apparent.

The reduction in aldosterone levels with age has been demonstrated in animal models [25] and humans [13,26]. Both impaired conversion of steroidogenic precursors to aldosterone in zona glomerulosa cells – although this finding is limited to an experimental study on ovariectomized rats [27] – and reduced adrenal responsiveness to angiotensin II [28] have been advocated as possible causes of this phenomenon. In our population, T allele carriers showed the 'physiological' decline of plasma aldosterone with age, accompanied by concomitant changes in serum electrolyte levels. In contrast, no such evidence was found in the CC homozygotes.

In summary, CC homozygosis characterized a subset of population in whom ageing is associated with little or no changes in phenotypic variables such as diastolic blood pressure and plasma aldosterone levels.

Although these results should be considered hypothesis-generating and should be confirmed by further analysis in independent samples, several considerations argue against the possibility that these findings are attributable to a type 1 error. First, the inter-individual variability of the parameters under consideration is large enough to allow for an accurate phenotypic characterization of the participants. Individuals on pharmacological treatment for hypertension were excluded from the analysis, thus limiting confounding by drug treatment. An attempt was also made to characterize the dietary intake of sodium and potassium. Although the value of surrogate indicators of dietary intake such as those used in our study is limited, the influence of these variables has not been considered at all in the studies so far published. Second, in a sample of 420 young normotensive subjects a trend to higher blood pressure levels was observed in CC homozygotes as compared with T carriers (R. Sarzani *et al.*, unpublished data, 2000). Moreover, a recent cross-sectional study on a Japanese population indicated that CC homozygosis was associated with an age- and gender-dependent nocturnal decline in blood pressure [18]. These findings indirectly confirmed that this genetic variant might act differently on blood pressure regulation over different age ranges. Third, in our study cross-sectional data are corroborated by longitudinal findings confirming the different age-related behaviour of diastolic blood pressure in CC or TC+TT subjects in long-term follow-up.

Some limitations of the present work are inherent to the nature of the Olivetti study cohort, which comprised only white male participants and may thus not be regarded as representative of the general population. For these reasons, the results of this study can be only generalized to a comparable white male population. Finally, the measurement of plasma renin activity – not performed at time of the study – might have been useful to gain further insight into the possible mechanism of the phenomena observed.

There are so far few data on possible functional expressions of the C(−344)T polymorphism of the *CYP11B2*. *CYP11B2* activity in the zona glomerulosa is controlled primarily by angiotensin II and potassium [29]. The C/T substitution occurs in the 5′-flanking region of the gene, within a putative binding site for the steroidogenic binding factor, SF-1: thus the variant could result in different transcription rates of the gene [30]. It might be speculated that the (−344)C allele confers a lower sensitivity to age-modulated factors affecting gene transcription, thus blunting the decline of aldosterone secretion with age.

In conclusion, while our findings appear to exclude that the C(−344)T polymorphism of the *CYP11B2* has a direct influence in 'setting' a basic level of blood pressure in our population, they support a role for this genetic variant in modulating factors affecting blood pressure variations around this set point, and suggest that environmental and individual backgrounds should be taken into account when evaluating the influence of genes on multifactorial diseases such as arterial hypertension.

Acknowledgements

We thank Dr A. Scottoni, Dr U. Candura and Ms M. Bartolomei for their support in organizing and coordinating work in the field, and the workers of the Olivetti factories for their co-operation. We acknowledge the hard work in the field of Drs E. Ragone, F. Stinga, L. Russo. Editorial help in manuscript revision by Mrs Rosanna Scala is gratefully acknowledged.

References

1 Corvol P, Persu A, Gimenez-Roqueplo AP, Jeunemaitre X. Seven lessons from two candidate genes in human essential hypertension: angiotensinogen and epithelial sodium channel. *Hypertension* 1999; **33**:1324–1331.
2 Munroe PB, Caulfield MJ. Genetics of hypertension. *Curr Opin Genetics Develop* 2000; **10**:325–329.
3 Lifton RP, Dluhy RG, Powers M, Rich GM, Cook S, Ulick S, *et al.* A chimaeric 11 beta-hydroxylase/aldosterone synthase gene causes gluco-

corticoid-remediable aldosteronism and human hypertension. *Nature* 1992; **355**:262–265.
4 Brand E, Chatelain N, Mulatero P, Fery I, Curnow K, Jeunemaitre X, *et al*. Structural analysis and evaluation of the aldosterone synthase gene in hypertension. *Hypertension* 1998; **32**:198–204.
5 Tamaki S, Iwai N, Tsujita Y, Kinoshita M. Genetic polymorphism of CYP11B2 gene and hypertension in Japanese. *Hypertension* 1999; **33**:266–270.
6 Davies E, Holloway CD, Ingram MC, Inglis GC, Friel EC, Morrison C, *et al*. Aldosterone excretion rate and blood pressure in essential hypertension are related to polymorphic differences in the aldosterone synthase gene CYP11B2. *Hypertension* 1999; **33**:703–707.
7 Komiya I, Yamada T, Takara M, Asawa T, Shimabukuro M, Nishimori T, *et al*. Lys(173)Arg and −344T/C variants of CYP11B2 in Japanese patients with low-renin hypertension. *Hypertension* 2000; **35**:699–703.
8 Paillard F, Chansel D, Brand E, Benetos A, Thomas F, Czekalski S, *et al*. Genotype–phenotype relationships for the renin–angiotensin–aldosterone system in a normal population. *Hypertension* 1999; **34**: 423–429.
9 Pojoga L, Gautier S, Blanc H, Guyene TT, Poirier O, Cambien F, *et al*. Genetic determination of plasma aldosterone levels in essential hypertension. *Am J Hypertens* 1998; **11**:856–860.
10 Brand E, Schorr U, Ringel J, Beige J, Distler A, Sharma AM. Aldosterone synthase gene (CYP11B2) C−344T polymorphism in Caucasians from the Berlin Salt-Sensitivity Trial (BeSST). *J Hypertens* 1999; **17**: 1563–1567.
11 Schunkert H, Hengstenberg C, Holmer SR, Broeckel U, Luchner A, Muscholl MW, *et al*. Lack of association between a polymorphism of the aldosterone synthase gene and left ventricular structure. *Circulation* 1999; **99**:2255–2260.
12 National Health Survey Series 11, No. 234. Blood pressure levels in persons 18–74 years of age in 1976–80, and trends in blood pressure from 1960 to 1980 in the United States. DHHS Publication (PHS) 86–1684; 1986.
13 Hegstad R, Brown RD, Jiang NS, Kao P, Weinshilboum RM, Strong C, *et al*. Aging and aldosterone. *Am J Med* 1983; **74**:442–448.
14 Stamler R, Stamler J, Riedlinger WF, Algera G, Roberts RH. Weight and blood pressure. Findings in hypertension screening of 1 million Americans. *JAMA* 1978; **240**:1607–1610.
15 Goodfriend TL, Egan BM, Kelley DE. Aldosterone in obesity. *Endocr Res* 1998; **24**:789–796.
16 Elliott P, Stamler J, Nichols R, Dyer AR, Stamler R, Kesteloot H, *et al*. Intersalt revisited: further analyses of 24 hour sodium excretion and blood pressure within and across populations. *BMJ* 1996; **312**:1249–1253.
17 Holland OB, Carr B. Modulation of aldosterone synthase messenger ribonucleic acid levels by dietary sodium and potassium and by adrenocorticotropin. *Endocrinology* 1993; **132**:2666–2673.
18 Matsubara M, Kikuya M, Ohkubo T, Metoki H, Omori F, Fujiwara T, *et al*. Aldosterone synthase gene (CYP11B2) C−344T polymorphism, ambulatory blood pressure and nocturnal decline in blood pressure in the general Japanese population: the Ohasama Study. *J Hypertens* 2001; **19**:2179–2184.
19 Kupari M, Hautanen A, Lankinen L, Koskinen P, Virolainen J, Nikkila H, *et al*. Associations between human aldosterone synthase (CYP11B2) gene polymorphisms and left ventricular size, mass, and function. *Circulation* 1998; **97**:569–575.
20 Ylitalo A, Airaksinen KE, Hautanen A, Kupari M, Carson M, Virolainen J, *et al*. Baroflex sensitivity and variants of the renin angiotensin system genes. *J Am Coll Cardiol* 2000; **35**:194–200.
21 Cappuccio FP, Strazzullo P, Farinaro E, Trevisan M. Uric acid metabolism and tubular sodium handling: results from a population-based study. *JAMA* 1993; **270**:354–359.
22 Strazzullo P, Iacone R, Siani A, Cappuccio FP, Russo O, Barba G, *et al*. Relationship of the Trp64Arg polymorphism of the beta3-adrenoceptor gene to central adiposity and high blood pressure: interaction with age. Cross-sectional and longitudinal findings of the Olivetti Prospective Heart Study. *J Hypertens* 2001; **19**:399–406.
23 Lahiri DK, Numberger JJ. A rapid non-enzymatic method for preparation of HMW DNA from blood for RFLP studies. *Nucl Acids Res* 1981; **19**:5444–5447.
24 Intersalt Cooperative Research Group. Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. *BMJ* 1988; **297**:319–328.
25 Jover B, Dupont M, Geelen G, Wahba W, Mimran A, Corman B. Renal and systemic adaptation to sodium restriction in aging rats. *Am J Physiol* 1993; **264**:R833–838.
26 Weidmann P, De Myttenaere-Bursztein S, Maxwell MH, de Lima J. Effect on aging on plasma renin and aldosterone in normal man. *Kidney Int* 1975; **8**:325–333.
27 Kau MM, Chen JJ, Wang SW, Cho WL, Wang PS. Age-related impairment of aldosterone secretion in zona glomerulosa cells of ovariectomized rats. *J Investig Med* 1999; **47**:425–432.
28 Belmin J, Levy BI, Michel JB. Changes in the renin–angiotensin–aldosterone axis in later life. *Drugs Aging* 1994; **5**:391–400.
29 Clyne CD, Zhang Y, Slutsker L, Mathis JM, White PC, Rainey WE. Angiotensin II and potassium regulate human CYP11B2 transcription through common cis-elements. *Mol Endocrinol* 1997; **11**:638–649.
30 White PC, Slutsker L. Haplotype analysis of CYP11B2. *Endocr Res* 1995; **21**:437–442.