

# Renin–angiotensin system gene polymorphisms: relationship with blood pressure and microalbuminuria in telmisartan-treated hypertensive patients

J Redon<sup>1</sup>  
 M Luque-Otero<sup>2</sup>  
 N Martell<sup>2</sup>  
 FJ Chaves<sup>1</sup>, on behalf of the  
 POLPRI investigators<sup>3</sup>

<sup>1</sup>Hypertension Clinic, Hospital Clinico, University of Valencia, Spain; <sup>2</sup>Hypertensión Unit, Hospital Clínico Universitario San Carlos, Madrid, Spain

**Correspondence:**  
 Professor J Redon, Hipertensión Unit,  
 Hospital Clínico Universitario, Avda. Blasco  
 Ibáñez, 14, Valencia 46010, Spain.  
 Tel: + 34 96 386 2647  
 Fax: + 34 96 386 2647  
 E-mail: josep.redon@uv.es

<sup>3</sup>For list of researchers of the POLPRI study see Appendix A1.

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## ABSTRACT

This study analyzed the relationship between four renin–angiotensin system (RAS) gene polymorphisms and the response to blood pressure lowering and development of microalbuminuria in 206 patients with essential hypertension treated once daily for 12 months with telmisartan 80 mg. Seated cuff blood pressure and urinary albumin excretion (UAE) were measured throughout the study. Patients were screened for the presence of the A-6G variant of the angiotensinogen gene, angiotensin-converting enzyme insertion/deletion polymorphism, and the A1166C and C573T polymorphisms of the angiotensin II type 1 receptor gene. No significant association was found between the presence of any gene polymorphism and the reduction of blood or UAE following telmisartan treatment. The results indicate that these RAS gene polymorphisms do not affect the antihypertensive activity and renoprotection in mild-to-moderate hypertensive patients treated with telmisartan.

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## INTRODUCTION

The blood pressure lowering responses of different antihypertensive agents are diverse and hard to predict in individuals, making prescribing a particular challenge. The Department of Veterans Affairs Cooperative Study on Anti-hypertensive Agents showed that only about half of the patients responded satisfactorily to antihypertensive monotherapy with any of six different agents from different therapeutic classes.<sup>1,2</sup> In this study and other similar ones, the magnitude of response varied by  $\pm 10$  mmHg, and in up to 20% of patients there was a paradoxical increase in blood pressure.<sup>3–5</sup> Furthermore, response to one class of antihypertensive agents did not reliably predict the efficacy of another drug with a different mechanism of action.<sup>6</sup>

The search for possible markers that can predict the blood pressure response before drug administration has been met with only limited success. Although some general trends have been identified with respect to race (eg African-Americans tend to respond better to diuretics and calcium channel blockers

than to  $\beta$ -blockers or angiotensin receptor blockers), other demographic traits, such as age, gender or body size, have no value in predicting blood pressure response.<sup>6</sup> The use of renin profiling to predict blood pressure-lowering response, whereby patients with high renin levels are treated with  $\beta$ -blockers or angiotensin-converting (ACE) inhibitors and those with low renin levels are given diuretics, has not proven as useful as was first hoped.<sup>7</sup> Other biochemical markers, such as insulin resistance, have similarly failed to show a pertinent relationship with hypertensive response.<sup>7</sup>

Investigation of the genetic contribution to hypertension, by examining mutations (or polymorphisms) in candidate genes, has expanded our knowledge of the mechanisms involved in the development of high blood pressure and hypertension-induced organ damage. The most studied candidate genes are those coding for the renin-angiotensin system (RAS). The RAS plays a central role in the pathophysiology of vascular disease,<sup>8</sup> and the genes that regulate the system may contribute to the development of hypertension and end-organ damage.<sup>9</sup> The major active peptide of the RAS is angiotensin II. Produced from the precursor molecule, angiotensinogen (AGT), via an enzyme cascade involving ACE enzyme, angiotensin II exerts numerous effects on the homeostatic regulation of blood pressure, the vast majority of which are mediated via the angiotensin II type 1 receptor (AT1R).<sup>9</sup>

The presence of polymorphisms in the *AGT*, *ACE* and *AT1R* genes of the RAS has been associated with adverse cardiovascular changes. For example, the presence of the A-6G polymorphism of the *AGT* gene has been linked with increased body weight gain in hypertensive patients;<sup>10</sup> the insertion/deletion (I/D) polymorphisms of the *ACE* gene have been associated with increased blood pressure, urinary albumin excretion (UAE) and target-organ damage in hypertensive patients;<sup>11</sup> the A1166C polymorphism of the *AT1R* gene has been associated with hypertension, left ventricular hypertrophy, aortic stiffness and exaggerated vasoconstriction, whereas the C573T polymorphism in the same gene appears to confer protection against the development of microalbuminuria in patients with hypertension.<sup>9</sup>

The role of genetic traits in predicting the response to antihypertensive drugs has been the subject of much research. Whether or not the response to treatment can be predicted from the presence of specific alleles of candidate genes has been the focus of several studies with heterogeneous results. The purpose of the present study was to evaluate the impact of four RAS gene polymorphisms on the antihypertensive response and development of microalbuminuria in patients receiving the angiotensin II receptor blocker telmisartan. The polymorphisms investigated were: A-6G, the A for G substitution of the *AGT* gene 6 nucleotides upstream from the start site; the *ACE* I/D polymorphism corresponding to an insertion or deletion of a 287 bp alu repeat, and two polymorphisms of the *AT1R* gene, A1166C and C573T, both in the noncoding part of exon 5.

## RESULTS

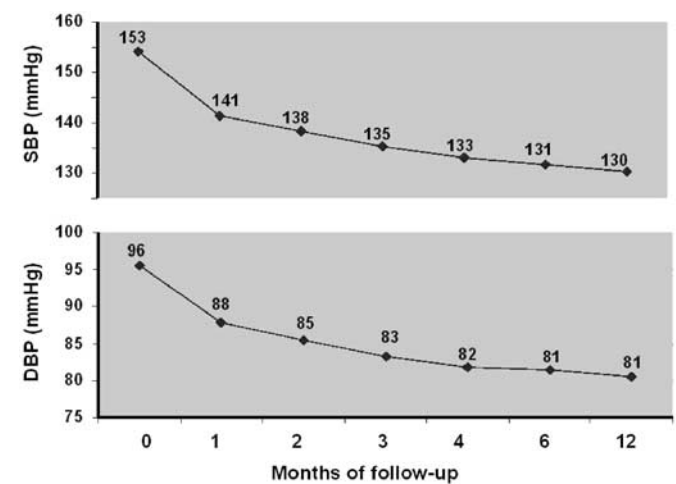
A total of 206 patients completed the study. The baseline characteristics of the patients in the study were consistent with those expected for a hypertensive Caucasian population. The patients all had mild-to-moderate hypertension. The mean body mass index (BMI) of patients in the study was 28.4 kg/m<sup>2</sup>, which is within the clinical definition of overweight (27–30 kg/m<sup>2</sup>), and 23% were defined as obese (BMI > 30 kg/m<sup>2</sup>). The mean UAE for the study population was 32.7 ± 84.0 mg/24 h. Of the study population, 28% had microalbuminuria (defined as UAE > 30 mg/24 h) at baseline (Table 1).

During the study, 81 patients (39%) required additional antihypertensive medication to achieve target blood pressure (<140/90 mmHg). Telmisartan-based treatment reduced both systolic blood pressure (SBP) and diastolic blood pressure (DBP) (Figure 1). Between baseline and the end of the 12-month study, SBP was significantly reduced by 23.9 mmHg ( $P < 0.001$ ) and DBP by 15.6 mmHg ( $P < 0.001$ ).

**Table 1** Baseline characteristics of the study population

Characteristic	Male	Female	Total
Number	125	81	206
Age (years)	62.1 ± 13.4	60.9 ± 13.6	61.0 ± 11.4
BMI (kg/m <sup>2</sup> )	29.9 ± 4.9	27.6 ± 3.8	28.4 ± 4.3
SBP (mmHg)	154.1 ± 13.0	153.1 ± 11.9	153.6 ± 12.6
DBP (mmHg)	96.1 ± 7.4	95.2 ± 6.8	95.2 ± 7.0
Heart rate (beats/min)	77.2 ± 9.9	74.9 ± 8.9	76.9 ± 9.3
Creatinine (mg/dl)	0.99 ± 0.21	0.95 ± 0.19	0.96 ± 0.20
Urinary albumin excretion (mg/24 h)	33.4 ± 80.0	31.4 ± 90.3	32.7 ± 84.0

BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure. Values are mean ± SD.



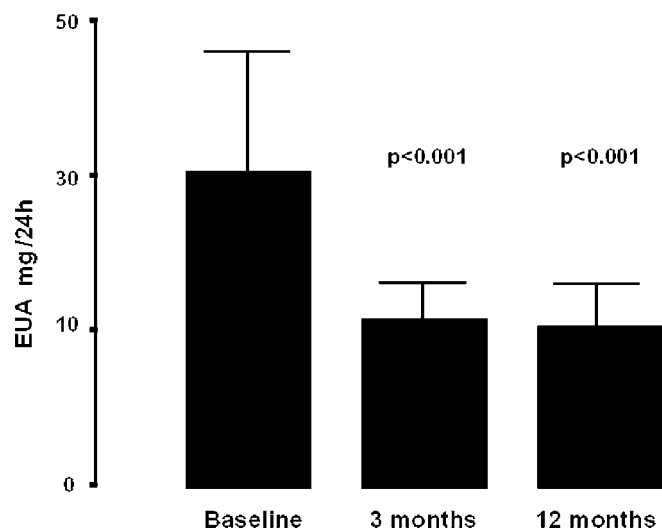
**Figure 1** Mean changes from baseline in SBP and DBP during 12 months treatment with telmisartan 80 mg once daily.

The reduction in blood pressure was seen as early as 1 month after starting therapy, achieved significance ( $P < 0.05$ ) and was maintained throughout the course of the study.

Telmisartan treatment also reduced UAE (Figure 2). This significant effect, which was apparent after 3 months of treatment ( $P < 0.001$ ), persisted to the end of the study.

#### Association of Polymorphisms with Blood Pressure or UAE

Genotype and allele distribution of the study population is summarized in Table 2. The Hardy–Weinberg equilibrium was maintained for the four polymorphisms analyzed. At baseline, SBP and DBP did not differ significantly for the genotypes for each of the analyzed polymorphisms, although SBP tended to be highest in patients carrying the A allele of the A-6G polymorphism of the *AGT* or the C allele of the A1166C polymorphism of the *AT1R* (Table 3).



**Figure 2** Mean change from baseline in UAE after 3 and 12 months of treatment with telmisartan 80mg once daily. Values are average  $\pm$  standard error.

Likewise, UAE did not differ among genotypes of the analyzed polymorphisms (Table 3).

Blood pressure values did not differ among the genotypes of each of the polymorphisms analyzed at 3 months nor at the year of the antihypertensive treatment (Table 3). The UAE values after 1 year of treatment were not different among the genotypes analyzed. The study was repeated using a dominant or recessive model and no differences were observed for the SBP, DBP or UAE changes during treatment. Multiple regression analysis of the A-6G, ACE I/D, A1166C and C573T polymorphisms revealed no significant association of any polymorphism with the observed changes in DBP, SBP or UAE (Table 4).

#### DISCUSSION

In this study, telmisartan treatment was associated with a significant reduction in SBP, DBP and UAE. Our analysis of polymorphisms in the *AGT*, *ACE* and *AT1R* genes of the RAS found no relationship between the presence of polymorphisms and the treatment-induced reduction in either blood pressure or UAE.

Previous studies have revealed heterogeneous relationships between RAS gene polymorphisms and hypertension or cardiovascular damage.

In the case of the A-6G polymorphism, there is very little published research except that the presence of the homozygous AA genotype is associated with increased body weight in hypertensives.<sup>10</sup> In the present study, 23% of patients were within the clinical definition of obesity and of these, 20% were homozygous for the A allele of the A-6G variant. In our study, no association was detected between the A-6G variant and the blood pressure lowering of telmisartan, suggesting that, on its own, this allele has no function in this setting.

The relationship of the ACE I/D polymorphism in the response of blood pressure lowering by antihypertensives has been the subject of many studies, but different investigators have reported divergent results. Dudley *et al*<sup>12</sup> found no association between the I/D polymorphisms and the blood pressure control achieved by a  $\beta$ -blocker

**Table 2** Distribution of the A-6G variant of the *AGT* gene, *ACE* enzyme I/D polymorphisms, and the A1166C and C573T polymorphism of the *AT1R* gene of the study population

Polymorphism	Genotype				
Allele I/D ( <i>ACE</i> )	II 23 (11.2%)	ID 84 (40.8%)	DD 99 (48.1%)	I 0.32 (0.2%)	D 0.68 (0.3%)
Allele A-6G ( <i>AGT</i> )	AA 39 (18.9%)	AG 68 (33.0%)	GG 99 (48.1%)	A 0.35 (0.2%)	G 0.65 (0.3%)
Allele A1166C ( <i>AT1R</i> )	AA 110 (53.4%)	AC 75 (36.4%)	CC 21 (10.2%)	A 0.72 (0.3%)	C 0.28 (0.1%)
Allele C573T ( <i>AT1R</i> )	CC 47 (22.8%)	CT 91 (44.2%)	TT 68 (33.0%)	C 0.45 (0.2%)	T 0.55 (0.3%)

**Table 3** Initial blood pressure values and UAE in each of the genotypes of the A-6G variant of the AGT gene, ACE enzyme I/D polymorphisms, and the A1166C and C573T polymorphism of the AT1R gene of the study population

Polymorphism	Systolic BP (mmHg)		Diastolic BP (mmHg)		UAE (mg/24 h)	
	Baseline	1 Year	Baseline	1 Year	Baseline	1 Year
<i>I/D ACE</i>						
II	153.7±16.1	131.6±14.2	94.4±6.5	80.3±7.2	26.4±29.1	11.4±9.4
ID	153.8±10.4	130.2±12.1	94.4±7.0	79.2±8.1	32.4±38.1	9.3±10.1
DD	153.4±13.2	130.1±14.1	96.2±6.1	82.1±6.9	34.2±44.0	12.3±14.1
<i>A-6G AGT</i>						
AA	153.6±11.9	130.3±13.1	95.2±6.3	80.4±8.2	34.4±36.1	9.8±10.1
AG	157.8±11.8	132.0±15.6	94.8±6.9	79.3±7.9	30.6±40.2	10.9±8.4
GG	149.6±11.7	130.4±14.3	96.0±6.9	81.4±8.1	32.5±38.3	11.3±6.2
<i>A1166C AT1R</i>						
AA	151.9±12.7	129.4±13.2	95.4±5.4	80.9±7.4	33.9±40.1	10.8±10.1
AC	154.0±12.8	131.2±15.8	95.7±6.0	79.4±8.5	36.1±38.2	11.3±9.2
CC	157.0±8.6	133.0±14.2	96.3±9.7	81.3±9.1	28.4±29.8	9.3±7.2
<i>C573T AT1R</i>						
CC	155.8±13.7	131.9±14.2	94.8±4.6	79.9±6.9	31.2±27.9	10.4±9.1
CT	151.0±12.8	129.3±12.0	94.7±7.6	80.1±7.2	32.6±38.3	12.3±10.1
TT	155.0±11.2	132.3±15.2	96.5±6.9	81.3±8.1	35.2±35.1	9.8±12.1

**Table 4** Significance (*P*-values) in a lineal regression analysis of the relationship between A-6G of AGT, I/D of ACE, and A1166C and C573T of AT1R polymorphisms and changes in SBP, DBP and UAE during the study

	A-6G	I/D	A1166C	T573C
SBP	0.4218	0.5653	0.6051	0.2704
DBP	0.5003	0.8162	0.2285	0.4549
UAE	0.5212	0.7104	0.5177	0.5845

(atenolol), an ACE inhibitor (lisinopril), or a calcium channel blocker (nifedipine sustained release) in previously untreated hypertensive patients. Similarly, Mondorf *et al*<sup>13</sup> were unable to identify a significant association between the presence of the ACE I/D variant and either hypertension or the blood pressure-lowering response of another ACE inhibitor, captopril. In contrast, a more recent study did show a relationship between the homozygous ACE II variant and the response to blood pressure lowering with the angiotensin II receptor blocker irbesartan.<sup>14</sup> The absence of association observed in the present study, in contrast to the previous one,<sup>14</sup> indicates that even though genetic background may contribute to drug responses, there are many other factors that mask it.

Despite gene linkage studies showing an association between primary hypertension and polymorphisms in the vicinity of the A1166C allele of *AT1R*,<sup>15</sup> previous clinical work has yielded similar results to those presented here, namely, that there appears to be no association between the

A1166C variant of *AT1R* and blood pressure response.<sup>14</sup> Kurland *et al*<sup>14</sup> found no association between the anti-hypertensive effect of irbesartan and the presence of the A1166C allele. This finding is consistent with those of other studies in healthy subjects and hypertensive patients who have shown that the presence of the A1166C genotype is unrelated to the increase in blood pressure following angiotensin II infusion.<sup>16–18</sup> Interestingly, there is some evidence to suggest that the A1166C allele may be responsible for increasing the sensitivity of the blood pressure response to low levels of angiotensin II rather than increasing total reactivity to the peptide.<sup>18</sup>

The presence of the C573T polymorphism of the *AT1R* gene has been associated with conferring a protective effect against the development of microalbuminuria in patients with essential hypertension. Chaves *et al*<sup>9</sup> demonstrated that untreated hypertensive patients with the TT genotype of this polymorphism consistently had the lowest UAE throughout the range of blood pressure values. Our results showed no relationship between the presence of the C573T polymorphism and the rate of UAE while on telmisartan therapy.

There were two limitations to our study that could have influenced the results. Firstly, our study did not examine the possibility that the association of polymorphisms with blood pressure response is context sensitive. For instance, we did not investigate how combinations of RAS gene polymorphisms might affect the blood pressure response to telmisartan. Current evidence suggests that the effects of some RAS gene polymorphisms are only detectable when present in a combination. For example, the combination of

the A1166C and the A-6G polymorphisms is associated with a high risk of hypertension in women,<sup>19</sup> and the ACE DD genotype plus the A1166C polymorphism is associated with the high blood pressure values in healthy subjects<sup>20</sup> and the presence of coronary artery disease in the general population.<sup>21</sup> The effects of gender, age, race or obesity on the distribution of polymorphisms and their effect on blood pressure response was also unexplored. Evidence is accumulating that some RAS gene polymorphisms may assume importance in the context of specific demographic traits, but the relationships are complex.<sup>19,22</sup> For instance, in hypertensive patients whose blood pressure is resistant to treatment, a significant association has been found between the presence of the A1166C allele and elevated SBP, but only in older and overweight patients.<sup>22</sup>

In conclusion, our study found no significant association between four gene polymorphisms of the RAS and the response to treatment with the angiotensin II receptor blocker telmisartan, neither in terms of antihypertensive activity nor in terms of developing microalbuminuria, in patients with mild-to-moderate hypertension. Our findings do not preclude the possibility of finding relationships between multiple gene polymorphisms or between polymorphisms within specific demographic traits, and blood pressure response; this area would appear to justify further investigation.

## MATERIAL AND METHODS

### Patient Population

Male and female patients >18 years of age who were diagnosed with essential mild-to-moderate hypertension of  $\geq 3$  months duration were included in the study. Mild-to-moderate (stage I or II) hypertension was diagnosed according to the criteria of the sixth report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC-VI)<sup>23</sup> (ie SBP 140–179 mmHg and DBP 90–109 mmHg). Patients with stage III hypertension (ie SBP  $\geq 180$  mmHg, DBP  $\geq 110$  mmHg), secondary or malignant hypertension, target-organ disease, heart failure class III or IV according to the New York Heart Association definition, arrhythmia, coronary disease or stroke within previous 6 months, chronic kidney or liver failure, and/or uncontrolled diabetes mellitus were excluded. Other grounds for exclusion included chronic treatment with nonsteroidal anti-inflammatory drugs or nitrates, an arm circumference >40 cm, prior to drug or alcohol abuse, intolerance to angiotensin II receptor blockers, calcium channel blockers, hydrochlorothiazide or lactose, or receipt of investigational treatment within the previous 3 months. No women of childbearing potential were eligible. Written informed consent was obtained from each patient before being included in the clinical trial, and patient identity was kept strictly confidential. Specific form consent was requested for genetic testing permission.

### Study Design

This prospective, multicenter clinical trial was conducted in compliance with the principles of Good Clinical Practice,

Declaration of Helsinki and local legislation. The initial 2-week run-in period was followed by a consecutive 54-week treatment period with telmisartan 80 mg once daily. If during active treatment SBP/DBP values remained >140/90 mmHg, additional antihypertensive treatment with the calcium channel blocker lecanidipine and/or hydrochlorothiazide was administered to attain the blood pressure goal of  $\leq 135/85$  mmHg. The genetic tests to be performed were prespecified at the time of the study design.

### Blood Pressure Measurement

Blood pressure was measured using a mercury sphygmomanometer in the seated position after 5 min of rest in a quiet environment, following recommendations by the British Hypertension Society.<sup>24</sup> SBP and DBP (Korotkoff phase I and phase V, respectively) were determined as the average of three measurements taken 5 min apart. Values were determined at the end of the run-in period (baseline), and then after 1, 2, 3, 4, 6 and 12 months of telmisartan 80 mg treatment, measurement was performed 24 h after dosing to provide trough values.

### Urinary Albumin Excretion

UAE was determined by two separate 24-h urine collections using an immunonephelometric assay (Behring Institute, limit of detection, 0.1 mg/dl; Inter- and intra-assay variability variation coefficients 2 and 7%, respectively; normal values for a healthy normotensive population of the same age range and sex was  $4.6 \pm 6.1$  mg/24 h). Aliquots of urine were taken from the 24-h and from the night time collections, stored in glass tubes at 4°C and analyzed 1–7 days after collection. For each patient, the UAE was considered as the mean of values obtained in the two separate 24-h urine collections. If the difference between the two samples was found to be >25% of the higher value, or a creatinine excretion over 24 h was lower than expected for body size and gender, a third sample was requested and analyzed. Microalbuminuria was defined as UAE 30–300 mg/24 h in the two samples. UAE was measured at baseline and again after 3 and 12 months of treatment.

### Clinical Efficacy End points

The primary efficacy end point was the reduction in DBP between baseline and the end of the 12-month active treatment period. Secondary efficacy end points included reduction in SBP, the proportion of patients needing additional treatment with a calcium channel blocker and/or hydrochlorothiazide and the reduction in UAE. Safety variables included the incidence of clinical adverse events and laboratory abnormalities experienced by the patients at different times of the study.

### Analysis of Polymorphisms (ACE I/D, A-6G, A1166C and C573T)

A blood sample for polymorphism analysis was obtained from each patient in the morning after a minimum of 8 h fasting at the time of randomization. Genomic DNA was

extracted from white blood cells using silica gel polymer according to the procedure of Tilzer *et al.*<sup>25</sup>

The ACE I/D polymorphism was genotyped using DNA amplification with oligonucleotides as described by Rigat *et al.*<sup>26</sup> Reactions were conducted using DNA amplification in a final volume of 15  $\mu$ l containing 0.75  $\mu$ mol/l of each primer, 75  $\mu$ mol/l of each NTP, 2 ng/ $\mu$ l DNA, 1.5 mmol/l MgCl<sub>2</sub>, 75 mmol/l Tris-HCl (pH 9.0), 20 mmol/l (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 5 mmol/l KCl and 0.2 U/ $\mu$ l Netzyme DNA polymerase (Need, SL, Valencia, Spain). The DNA was amplified for 40 cycles with denaturation at 94°C for 90 s (PTC-100 thermal cycler, MI Research). The polymerase chain reaction (PCR) products underwent electrophoresis using 2% agarose gel. DNA was visualized with ethidium bromide staining.

The region of the *AT1R* located between nucleotides 423 and 1278 of the cDNA was amplified using oligonucleotides 5'-GGC TTT GCT TTG TCT TGT TG and 5'-AAT GCT TGT AGC CAA AGT CAC CT as sense and antisense primers, respectively. Amplification was conducted as described above. The A1166C and C573T polymorphisms of the *AT1R* gene were analyzed simultaneously by PCR using the technique of Chaves *et al.*<sup>9</sup> PCR product was digested with 0.5 U *FokI*, and the resultant fragments were analyzed by the single-strand conformation polymorphism (SSCP) analysis using 10  $\times$  10 cm<sup>2</sup> 10% acrylamide gel, 10% glycerol and 1  $\times$  TEB buffer. The total digestion volume was mixed with 5  $\mu$ l loading buffer (95% formamide, 20 mmol/l EDTA, 1% xylene cyanol and bromophenol blue). After denaturation (heated at 95°C for 2 min, then chilled on ice), DNA fragments underwent gel electrophoresis at 400 V for 3 h at 18°C. SSCP bands were correlated with the polymorphisms by direct PCR product sequencing.

The A-6G polymorphism located in the promoter region of the *AGT* gene was analyzed using the method described by Wang *et al.*<sup>27</sup> The 3' promoter region was amplified with oligonucleotides 5'-GAG GTC CCA GCG TGA GTG TCG C and 5'-CTC AGT TAC ATC CTG AGA GAG ACA AGA CC under standard PCR conditions. Fragments were analyzed by SSCP using water-cooled vertical minigels (10  $\times$  10 cm<sup>2</sup>). After adding 9  $\mu$ l loading buffer to 1  $\mu$ l PCR product, the mix was denatured and loaded into the 1  $\mu$ l gel. The electrophoresis conditions were the following: 14% acrylamide into 1  $\times$  TBE buffer at 400 V for 3 h at 15°C, after which the gels were silver stained.

### Statistical Methods

Considering that in the study several comparisons were included, the sample size calculation was performed on that with the lowest allele frequency expected and using a dominant model. The sample size of the study was calculated assuming a standard deviation for the DBP measurement of 9.9 mmHg; a difference to be detected between genotypes of at least 4 mmHg and using a bilateral Student's *t*-test with protection against type I error of 5% and against type II error of 80%. It was calculated that the main analysis of the study required 196 patients in total. Early withdrawals and major deviations were considered likely to affect 20% of included patients, so the sample size

necessary during initial enrollment was calculated to be 245 patients.

Data management and statistical analysis were conducted using SPSS for Windows 6.12. The analysis was conducted on a per-protocol basis that included those patients who were compliant with inclusion/exclusion criteria, had a valid final blood pressure and UAE measures and who completed DNA extraction. Baseline quantitative variables were described by arithmetic mean and standard deviation, and analyzed using either Student's *t*-test or analysis of variance (ANOVA). For baseline categorical variables, absolute and relative frequencies were described, and analyzed by  $\chi^2$  test or Fisher's exact test, as required.

The main efficacy analysis compared the DBP reduction from baseline by a two-tailed Student's *t*-test. Similar analyses were performed for SBP and UAE. The proportion of patients receiving more than one drug was also analyzed. Multiple regression analysis was performed to assess the factors related to the changes in UAE during the follow-up and related to the interaction of genotypes. The association between polymorphisms and UAE was examined using a codominant inheritance model of the alleles.

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### DUALITY OF INTEREST

None declared.

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## Appendix A1

### List of researchers of the POLPRI study

J Redon, V Giner, Hospital Clínico Universitario, Valencia; M Luque, N Martel, Hospital Clínico San Carlos, Madrid; C Fernandez-Torres, Hospital Virgen de las Nieves, Granada; S Najaty, Hospital Clínico Universitario, Valladolid; J Olivan, Hospital Virgen de la Macarena, Sevilla; A Martin-Hidalgo, Hospital General Universitario, Elche; P Cia, Hospital Clínico Universitario, Zaragoza; MA Courel, Hospital Xeral, Vigo; J Plana, Hospital San Camil, Barcelona; J Villatoro, Hospital General, Castellon; C Fernandez, Ciudad Sanitaria Virgen del Rocío, Sevilla; J Poblador, Hospital General, Mallorca; P Aranda, Hospital Regional Carlos Haya, Málaga; J Abellan, Centro de Salud, Murcia; J Viladoms, Hospital de Mollet de Vallés, Barcelona; A Martinez-Amenos, Hospital Principes de España, Hospitalet del Llobregat; A Liebana, Hospital Ciudad de Jaén, Jaen; A Salcedo, Hospital de Galdácano, Bilbao.