ACE gene insertion/deletion polymorphism modulates capillary permeability in hypertension

Giulia DELL’OMO*, Giuseppe PENNO†, Laura PUCCI†, Daniela LUCCHESI†, Carmen FOTINO†, Stefano DEL PRATO† and Roberto PEDRINELLI∗
∗Dipartimento Cardio Toracico, Università di Pisa, 56100 Pisa, Italy, and †Dipartimento di Endocrinologia e Metabolismo, Università di Pisa, 56100 Pisa, Italy

ABSTRACT

A D/D (deletion/deletion) polymorphism within the ACE (angiotensin 1-converting enzyme) gene increases the risk of microalbuminuria, a predictor of atherosclerotic vascular disease, in essential hypertension. It is unknown, however, whether this genetic profile is accompanied by disturbed macromolecular permeability of systemic capillary endothelium, possibly in the context of generalized endothelial dysfunction. In the present study, the ACE gene polymorphism was determined by PCR in 79 never-treated uncomplicated hypertensive men and 16 normotensive men as controls. Evaluation variables were TERalb (transcapillary escape rate of albumin; the 1-h decline rate of intravenous 125I-albumin, a measure of integrity of systemic capillary endothelium), albuminuria and forearm vasodilation to intra-arterial acetylcholine, an index of NO (nitric oxide)-mediated vasomotion, in addition to a series of sensitive parameters of albumin permeation (blood pressure, metabolic status and smoking habits). Analyses were done by comparing D/D homozygotes with grouped I/D (insertion/deletion) and I/I (insertion/insertion) subjects. TERalb was higher in D/D hypertensives, who had higher albuminuria, more frequent microalbuminuria and comparable forearm responsiveness to intra-arterial acetylcholine. Fasting glucose and insulin, insulin sensitivity, 24-h blood pressure, smoking habits and metabolic parameters did not differ between the two groups. TERalb and urine albumin values were positively associated in the hypertensive subjects. In conclusion, ACE D/D homozygosis, independently of several confounding factors, associates with higher TERalb in men with essential hypertension. This may reflect noxious genetic influences on systemic vascular permeability, a critical control mechanism for atherogenesis in the absence of grossly impaired NO-mediated arteriolar responsiveness. The parallel behaviour of TERalb and albuminuria suggests some shared genetically mediated determinant of renal and systemic microvascular abnormalities in hypertension.

Key words: ACE gene polymorphism, albuminuria, capillary permeability, endothelial function, hypertension, transcapillary escape rate of albumin.

Abbreviations: ACE, angiotensin 1-converting enzyme; ACh, acetylcholine; AUC, area under the curve; BMI, body mass index; BP, blood pressure; CI, confidence interval; DBP, diastolic blood pressure; D/D, deletion/deletion; FBF, forearm blood flow; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; I/D, insertion/deletion; I/I, insertion/insertion; LDL, low-density lipoprotein; MA, microalbuminuria; MetS, metabolic syndrome; NO, nitric oxide; OGTT, oral glucose tolerance test; OR, odds ratio; SBP, systolic blood pressure; SNP, sodium nitroprusside; TERalb, transcapillary escape rate of albumin; UAE, urinary albumin excretion.

Correspondence: Professor Roberto Pedrinelli (email r.pedrinelli@med.unipi.it).
INTRODUCTION

Consistent evidence supports the role of the ACE (angiotensin I-converting enzyme) I/D (insertion/deletion) polymorphism in the modulation of UAE (urinary albumin excretion) in non-diabetic patients with essential hypertension. Several previous studies (for example, [1–5]), in fact, have associated the presence of the D allele within the ACE gene with higher UAE and more frequent MA (microalbuminuria), a sign of subclinical renal damage and a predictor of atherosclerotic cardiovascular events [6], in hypertension. It is unknown, however, whether this noxious genetic influence on UAE, which may reflect more permeable renal glomeruli, is also accompanied by systemic changes in the permeability of capillary endothelium, a key determinant of endogenous defence against vascular injury and atherosclerosis [7,8].

To assess this possibility, we measured TERalb (transcapillary escape rate of albumin), a measure of the integrity of systemic capillary permeability [9], and its relationship with the ACE I/D genotype and UAE in a group of non-diabetic never-treated uncomplicated hypertensive men. To evaluate a possible co-existence with any abnormal function in arteriolar endothelium, we also investigated the association of the I/D genotype with any abnormal function in arteriolar endothelium, a key determinant of endogenous defence against vascular injury and atherosclerosis [7,8].

MATERIALS AND METHODS

Subjects

Seventy-nine never-treated sedentary Caucasian hypertensive men, apparently genetically unrelated, and 16 normotensive age-matched participants were included in the study. Exclusion criteria were diabetes (fasting plasma glucose levels >125 mg/dl, and/or post-glucose load >200 mg/dl), renal insufficiency (serum creatinine >1.4 mg/dl), proteinuria using the dipstick test and impaired cardiac function (ejection fraction <50 %). In all, co-existing coronary and vascular disease were excluded through medical history, physical examination, routine blood chemistry, baseline EKG or, where indicated, treadmill test, echocardiography, and carotid and lower limb echo-Doppler sonography.

In accordance with the Declaration of Helsinki and institutional guidelines, the local Ethical Committee approved the protocol, and subjects were aware of the investigational nature of the study and agreed to participate after providing written consent.

Determination of the ACE genotype

Whole blood was collected in EDTA tubes and stored at −20 °C until extraction. Genomic DNA was extracted by manual saline extraction from lysed white blood cells, as described previously [11]. The polymorphic region in intron 16 of the ACE gene (17q23) was amplified by PCR performed with 10 ng of DNA using 5 pmol of both forward (5′-CTGGAGACCATCCTCCATCTTCTTCTG-3′) and reverse (5′-GATGGTGCCATCACATTCTGTCAGAT-3′) primers. Since the amplification of the I allele is less efficient than the D allele, the specificity of D/D (deletion/deletion) genotyping was increased by amplifying all D/D samples again using a pair of primers specific for the I sequence (forward, 5′-TGGGACCACGGGCCGCATTAC-3′; and reverse, 5′-TCGCACGCCCCTCCCATGCCCCATAA-3′) in the presence of 5 % (v/v) DMSO. Two different operators, unaware of the patient’s clinical status, read the gels independently.

TERalb

TERalb studies were performed between 14.00 and 16.00 hours after a 4-h fast, with patients not consuming tea, coffee, alcohol or tobacco from early in the morning, as described previously [12]. In brief, 125I-labelled human serum albumin (6–8 μCi; 222–296 kBq; SARI-125 A-2; SORIN Biomedica) was obtained by using an electrolytic technique, a procedure that does not alter the biological behaviour of albumin in vivo. Free 125I was eluted by passage through a Sephadex G-25-M column (Column PD-10; Pharmacia), a purification step that decreased free 125I content in the injected dose to <1 %. Radiolabelled albumin was injected as a bolus after the subjects had rested for 30 min in a sitting position, and blood samples were taken from the contralateral arm every 5 min during the hour following injection. Radioactivity was measured (Cobra 5000 γ-counter; Packard) in duplicate in whole blood samples centrifuged for 10 min at 3000 g for 40 min. Haematocrit (Coulter Counter 55; Coulter Electronics) was determined in each sample. Serum albumin was measured by immunonephelometry (interassay variation co-efficient, 5.2 %; Laser Nephelometer System; Behring).

UAE

Urinary albumin was measured by nephelometry using a commercially available kit (0.1 mg/dl detection limit; Roche). Intra- and inter-assay variation co-efficients at different concentrations of urine albumin were: 2.7 % and 3.2 % (0.3 mg/dl), 2.4 % and 2.9 % (1 mg/dl), 1.9 % and 2.6 % (10 mg/dl), and 1.8 % and 3.2 % (30 mg/dl) respectively. To minimize the confounding influence of daily physical activity and to facilitate the collection procedure, urine was collected from 20.00 to 08.00 hours over 3 consecutive days. Urinary and serum creatinine was measured by standard colorimetric methods.

Forearm infusion

Forearm studies (n = 66 hypertensive patients, and n = 12 normotensive controls) were performed in the morning in a quiet air-conditioned room 24–48 h apart from
TERalb determination. Subjects fasted overnight and were instructed to refrain from heavy exercise and to avoid smoking and emotional excitement from the day before the experiment. A 22-gauge polyethylene catheter (Angiocath, Becton Dickinson) was inserted into the right brachial artery, the arterial line was connected to an infusion pump (Perfusor, Secura FT; Braun) and subjects were then allowed to rest for approx. 30 min. Total FBF (forearm blood flow) was measured by venous plethysmography with a strain-gauge apparatus (EC 5R Plethysmograph; Hokanson). Strands made of silastic tubing (0.4 mm inner diameter and 0.8 mm outer diameter) filled with mercury were used. The gauge was applied on the arm, 5–6 cm distal to the elbow, at a tension sufficient to keep the gauge in the same position throughout the experiment. The subject’s forearm was kept on a table, slightly flexed and inclined at approx. 45° to the horizontal plane with the wrist and hand supported by sand bags. At 1 min before FBF determination, a pneumatic paediatric cuff was placed around the wrist and inflated to suprasystolic arterial BP (blood pressure) in order to exclude the hand vascular region. A second cuff was placed proximal to the plethysmograph and automatically inflated to a pressure of 40 mmHg to allow FBF measurement, according to the venous occlusion method. BP was measured every 5 min throughout the study on the contralateral arm by an automated device (NIBP KO 7267.004; Kontron Instruments). Further details of the method have been published previously [13].

Fresh solutions of ACh (Miovisin™, Farmigea), an NO-releasing compound [10], and SNP (sodium nitroprusside; Nipride™; Malesci), used as an internal control for NO-independent mechanisms [14], were infused sequence was randomized, and a 30-min interval was left between the first and second drug administration. Preliminary studies had shown that these infusion periods were sufficient to reach a plateau of FBF response without changes in systemic arterial pressure and contralateral FBF. Local administration of ACh and SNP was preceded by sand bags. At 1 min before FBF determination, a pneumatic cuff was applied on the arm, 5–6 cm distal to the elbow, at a tension sufficient to keep the gauge in the same position.

Ancillary parameters
Office SBP and DBP (systolic and diastolic BP respectively) were the mean of several indirect recordings. A 24-h BP (08.30–08.00 hours) was measured using an oscillometric monitor (Diasys Integra; Novacor).

An OGTT (oral glucose tolerance test), using 75 g of glucose, was performed in the morning after an overnight fast. Following collection of baseline samples, specimens for plasma glucose and insulin were drawn 0.5, 1, 1.5 and 2 h after administration of the glucose load. Plasma glucose was measured by the gluco-oxidase method using a Beckman Glucose Analyser I/I (Beckman Instruments), and plasma insulin was measured using an immunoradiometric assay (Biosource; no cross-reactivity with human proinsulin) with an interassay variation coefficient of 5%.

Total cholesterol, HDL (high-density lipoprotein)- and LDL (low-density lipoprotein)-cholesterol and triacylglycerols (triglycerides) were assessed under fasting conditions by enzymatic colorimetric techniques (Roche).

Anthropometric measurements (height and weight) were taken after each participant had removed his shoes and upper garments. Body weight was measured to the nearest 0.1 kg on a scale with attached height measure (SECA 207).

Smoking status was defined as active smokers compared with non-smokers, without distinction between former and never smokers.

Data processing
Plasma 125I-albumin concentration (c.p.m./ml) was plotted on a semi-logarithmic scale, and TERalb (%/h) was calculated from the mono-exponential disappearance rate constant of the 125I curve from 10–60 min. Plasma volume (ml/1.73 m2) was determined by extrapolation to 0 time of the disappearance curve corrected for the injected dose of tracer obtained by weighing the syringes before and after injection [12].

UAE (µg/min) was the average of three consecutive overnight collections (mean variation coefficient of the triplicates, 22%). MA was defined as UAE ≥ 15 µg/min, an accepted threshold for overnight collection [15]. Because of its skewed distribution, albuminuria was log-transformed to normalize its distribution. Creatinine clearance (ml·min⁻¹·1.73 m²⁻¹) was calculated according to standard formulae [(12-h urine volume × urine creatine/serum creatine) × (1.73/body surface area)].

FBF (ml·100 ml⁻¹·forearm volume·min⁻¹) represents the mean of four or five determinations obtained over the last 2 min of each experimental period. Percentage FBF changes from baseline were the evaluation variable.

OGTT data were summarized as AUC (area under the curve; determined using the trapezoidal rule) values. Insulin sensitivity was assessed by HOMA-IR (homeostasis model assessment of insulin resistance) [16], using the equation:

\[
\text{HOMA-IR} = \frac{\text{[fasting serum insulin (µ-units/ml) }}{22.5}
\times \text{fasting plasma glucose (mmol/l)}
\]

Increasing HOMA-IR values denote progression from normal to impaired insulin sensitivity. BMI (body mass index) was calculated as weight/height² (kg/m²). MetS (metabolic syndrome) was diagnosed according to the National Cholesterol Education Program Adult Treatment Panel III [17] (elevated BP and at least two of the
Table 1 Comparison of hypertensive patients and normotensive controls
Values are means ± S.D., or geometric means (interquartile range). NS, not significant; N/A, not assessed.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotensive controls</th>
<th>Hypertensive patients</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>16</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>47 ± 12</td>
<td>48 ± 10</td>
<td>NS</td>
</tr>
<tr>
<td>ACE genotype (D/D; I/D; I/I)</td>
<td>6; 8; 2</td>
<td>36; 36; 7</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine clearance (ml·min⁻¹·1.73 m⁻²)</td>
<td>104 ± 29</td>
<td>110 ± 36</td>
<td>NS</td>
</tr>
<tr>
<td>Active smokers (n)</td>
<td>7 (44 %)</td>
<td>26 (33 %)</td>
<td>NS</td>
</tr>
<tr>
<td>Office SBP (mmHg)</td>
<td>126 ± 10</td>
<td>149 ± 14</td>
<td>N/A</td>
</tr>
<tr>
<td>Office DBP (mmHg)</td>
<td>76 ± 6</td>
<td>96 ± 11</td>
<td>N/A</td>
</tr>
<tr>
<td>24-h SBP (mmHg)</td>
<td>121 ± 7</td>
<td>137 ± 16</td>
<td>N/A</td>
</tr>
<tr>
<td>24-h DBP (mmHg)</td>
<td>75 ± 6</td>
<td>88 ± 9</td>
<td>N/A</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dl)</td>
<td>88 ± 12</td>
<td>98 ± 11</td>
<td>P = 0.002</td>
</tr>
<tr>
<td>Glucose AUC₀₋₁₂₀ min (µg·ml⁻¹·2 h⁻¹)</td>
<td>14.3 ± 2.1</td>
<td>16.1 ± 3.1</td>
<td>P = 0.02</td>
</tr>
<tr>
<td>Fasting insulin (µε-units/ml)</td>
<td>7.7 (2.3)</td>
<td>12.3 (10.4)</td>
<td>P = 0.004</td>
</tr>
<tr>
<td>Insulin AUC₀₋₁₂₀ min (µε-units·ml⁻¹·2 h⁻¹)</td>
<td>5.3 (6.1)</td>
<td>12.3 (10.4)</td>
<td>P = 0.014</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.7 (0.9)</td>
<td>2.9 (2.8)</td>
<td>P = 0.0013</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>46 ± 9</td>
<td>45 ± 14</td>
<td>NS</td>
</tr>
<tr>
<td>Serum triacylglycerols (mg/dl)</td>
<td>94 (42)</td>
<td>160 (124)</td>
<td>P = 0.004</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>24.1 ± 1.7</td>
<td>27.3 ± 3</td>
<td>P = 0.0001</td>
</tr>
<tr>
<td>MetS (n)</td>
<td>0 (0 %)</td>
<td>32 (41 %)</td>
<td>P = 0.005</td>
</tr>
<tr>
<td>Office DBP (mmHg)</td>
<td>193 ± 47</td>
<td>208 ± 42</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>127 ± 43</td>
<td>126 ± 38</td>
<td>NS</td>
</tr>
</tbody>
</table>

following: triacylglycerols ≥ 150 mg/dl, HDL-cholesterol < 40 mg/dl, fasting plasma glucose ≥ 110 mg/dl and BMI > 30 kg/m²).

Statistics
Appropriate ANOVA models and χ² statistics were used to test the statistical significance of continuous and categorical parameters respectively. Intra-individual association between variables was tested by correlation co-efficient analysis. The association of TERalb (coded as 1 for upper-quartile values, and 0 for the three remaining ones; cut-off, 11.1 %/h) with the ACE I/D genotype and other continuous and categorical covariates was analysed by multivariate logistic regression, and ORs (odds ratios) and 95 % CIs (confidence intervals) were used to estimate relative risks. Statistical significance was set at P < 0.05. Descriptive statistics are means ± S.D., or geometric means (interquartile range) for skewed parameters.

RESULTS
In the overall normotensive (n = 16) and hypertensive (n = 79) groups, 42 (44 %) subjects had a D/D genotype compared with 44 (46 %) and 9 (9 %) with an I/D and I/I (insertion/insertion) genotype respectively. Allelic distribution was in Hardy–Weinberg equilibrium (χ² < 0.271, degrees of freedom = 1; P > 0.6) and did not differ by BP status (Table 1). Because of their low number, making separate statistical comparisons meaningless, I/I subjects were grouped with I/D subjects in statistical analyses.

TERalb by ACE genotype
Fasting and stimulated glucose and insulin, HOMA-IR index, triacylglycerols, BMI and UAE were higher, and MetS more frequent, in hypertensive patients compared with normotensive controls (Table 1). When compared with normotensive controls, TERalb was higher in hypertensive patients, either as a whole (7.4 ± 1.5 %/h (n = 16) compared with 9.8 ± 2.4 %/h (n = 79); P = 0.0002) or when stratified by ACE genotype (D/D, 7.0 ± 2.7 %/h in normotensive controls (n = 36); P = 0.005); and I/D + I/I, 7.8 ± 1.1 %/h in normotensive controls (n = 10) compared with 9.3 ± 2.1 %/h in hypertensive patients (n = 43; P = 0.031).

In the hypertensive patients, TERalb was higher (P = 0.03) in D/D homozygotes than I/D + I/I subjects (Figure 1), independent of age, BP levels, metabolic parameters, renal function and MetS frequency, which were closely matched in the two hypertensive subgroups (Table 2). Haematocrit (38.3 ± 4.7 % in I/D + I/I subjects compared with 39.5 ± 3.5 % in D/D subjects), serum albumin (4.3 ± 0.3 g/dl in I/D + I/I subjects compared with 4.6 ± 0.3 g/dl in D/D subjects) and plasma volume (2837.3 ± 359.4 ml/1.73 m² in I/D + I/I subjects compared with 2900.5 ± 400.5 ml/1.73 m² in D/D subjects) did not differ.
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When TERalb (as a dichotomized variable; cutoff, 11.1 %/h) was used as the dependent variable in a multivariate logistic regression model, the allelic OR for ACE D/D compared with pooled I/D + I/I was 3.1 (95 % CI, 1.1–7.5; \( P = 0.04 \)) after accounting for BP, LDL-cholesterol, smoking status, the presence of MetS and HOMA-IR index.

UAE and FBF responses by ACE genotype
In D/D hypertensive homozygotes, UAE [12.1 (16) compared with 8.0 (7.3); \( P = 0.03 \)] was higher and MA (42 % compared with 14 %; \( P = 0.01 \)) was more frequent compared with I/D + I/I hypertensives. In the hypertensives as a whole, TERalb correlated positively with UAE \( (r = 0.23, P = 0.03; n = 79) \), but not office and 24-h BP, BMI, fasting and post-load plasma glucose and insulin, and HOMA-IR. No correlation was found among normotensive controls (Figure 2).

Baseline FBF [3.7 ± 1.3 ml · 100 ml \(^{-1}\) · min \(^{-1}\) of forearm volume · min \(^{-1}\) in I/D + I/I subjects \( (n = 35) \) compared with 3.6 ± 1.1 ml · 100 ml \(^{-1}\) · min \(^{-1}\) forearm volume · min \(^{-1}\) in D/D subjects \( (n = 31) \)] and FBF responses to intra-brachial ACh and SNP did not differ according to ACE genotype or by hyper- compared with normo-tensive status (Figure 3).

DISCUSSION
The major and original finding of this cross-sectional genetic association study in never-treated uncomplicated hypertensive men was the independent association between ACE D/D homozygosis and faster TERalb. The results are consistent with a more deranged endothelial barrier function at the level of the systemic capillary circuit, where most of the albumin permeation process takes place (for reviews, see [7–9]). Faster TERalb also co-existed with higher UAE, suggesting some common genetically mediated determinant for the abnormal behaviour of renal and systemic microvessels, without evidence of NO-mediated endothelial dysfunction of systemic arterioles, as assessed by forearm ACh stimulation.
Figure 3  FBF responses to ACh and SNP by ACE genotype in hypertensive patients
Values are means ± S.D. As a comparison, the Figure also contains results for control subjects. Open bars, controls (n = 12); grey bars, I/D + I/I genotype (n = 35); closed bars, D/D genotype (n = 31). For further details, see text.

Pathophysiological implications
Although the mechanistic links between the ACE polymorphism and a remote and complex phenotype such as capillary permeability cannot be elucidated, some plausible pathophysiological inferences may still be drawn from our results. First, whatever the underlying biological mechanism, the unfavourable influence of the ACE D/D genotype should not be seen as the cause, but rather as a modulating factor for the defective systemic capillary permeability featured in human hypertension [9,18,19] since, irrespective of the ACE genotype, TERab was higher in hypertensive patients than normotensive controls. One might also wonder whether the influence of the ACE D/D polymorphism extends to normotensive subjects, but the limited number of control subjects included in our sample makes this point immaterial.

Secondly, the effect of a series of sensitive parameters of capillary albumin permeation, such as elevated BP [19] and LDL-cholesterol [13], hyperinsulinemia [20], smoking habits [21] and MetS phenotype [22], could probably be excluded because of the homogeneous distribution of these parameters across the ACE genotypes. Atherosclerotic vascular disease also affects TERab [23], but our patients were carefully selected for the absence of clinical vascular disease. Given the above considerations, other possibilities should be taken into account, and the activation of the renin–angiotensin system appears to be a plausible candidate mechanism. In fact, D/D homozygosis is associated with higher circulating and tissue ACE levels [24], the rate-limiting step in biologically active angiotensin II production [25], and also stimulates its local production [26]. In turn, angiotensin II increases oxidative stress through NADH/NADPH oxidase stimulation and, in conjunction with other mediators, such as cytokines, NO, endothelin-1, prostaglandins and the Rho protein pathway, promotes vascular inflammation, increases capillary permeability and impairs endothelial function [27]. With regard to the potential impairment of endothelial function, it was of interest that the homogeneous forearm vasodilatory responsiveness to ACh gave a negative result, in agreement with previous reports [28], suggesting a different impact of ACE genotypic variants on the capillary endothelial cell monolayer localized at the blood–vessel interface and endothelial cells covering resistance-sized forearm arterioles. However, other possibilities cannot be excluded as the infused forearm model tests only endogenous NO bio-availability in response to ACh, a NO-releasing stimulus [10], whereas TERab measures systemic capillary permeability in basal conditions under the influence of several and interacting stimuli [7–9]. Furthermore, several heterogeneous organs and tissues contribute to the determination of transvascular albumin leakage, whereas forearm responses represent a single, mainly muscular, vascular bed. Finally, NO-mediated relaxation needs only endothelial synthesis and diffusion of the substance to the underlying smooth muscle cells, whereas capillary permeability requires a more complicated functional and structural interaction of cell junctions and extracellular matrix with endothelial cells [7–9]. On the other hand, the preserved forearm responsiveness to locally infused ACh, consistent with previous results in the forearm [29,30], coronary [31] and subcutaneous [32] arterioles, shows that impaired endothelium-mediated vasodilatation is not universal among essential hypertensive patients, but rather may develop at more complicated stages [33] or with longer duration of hypertensive disease [34]. However, we have no arguments in favour or against these possibilities as our present study did not address these specific issues.

Some comment should also be made with regard to the confirmed [1–5] association of higher UAE with the ACE D/D genotype and, more importantly in this context, its association with TERab, which suggests some shared genetically promoted determinant in the abnormal behaviour of renal and systemic microvessels in ACE D/D hypertensive patients. Previous findings have shown that more albumin leaks through exaggeratedly permeable glomeruli characterized by decreased size and charge selectivity [35], and that albuminuria may reflect a generalized transvascular albumin leakage in clinically healthy and diabetic subjects [36,37]. It should be noted, however, that the correlation between TERab and UAE,
albeit statistically significant, was rather weak in our present group of subjects. We were also unable to find a similar correlation in a previous series of hypertensive subjects [19], perhaps because the modulation of both parameters by multiple haemodynamic, metabolic and behavioural factors [6,8] may have obscured such a link. It may not be irrelevant to note that higher UAE predicts cardiovascular events even in non-hypertensive individuals [38], and MA is frequent among patients with mildly elevated BP, lean body size and no evidence of metabolic abnormalities and subclinical inflammation [39]. In these subsets, perhaps genetic influences may be more influential on the renal and systemic microvascular system; an attractive possibility to be tested in the future.

Limitations of the study
A first obvious limitation of our present study is with regard to the strength of conclusions based upon a sample size of 79 patients. However, calculations accounting for effect of size and allele frequency for predisposing alleles [40] indicate that, albeit limited, these numbers have enough statistical power to detect significant association when, as in our conditions, the homozygous susceptibility genotype has a large biological impact (allelic OR = 3.1) and the prevalence of the susceptibility allele in the control group is high (38%). On the other hand, we are aware that small initial studies frequently overestimate the true size effect, thus increasing sample size requirements to thousands of cases and controls (for example, [41]), a goal impossible to achieve when dealing with a laborious parameter such as TERalb. For this reason, our present study should be seen as a biologically plausible hypothesis-generating contribution awaiting support from larger trials. Secondly, we studied an all-male hypertensive group and the influence of the ACE I/D polymorphism may differ by gender [42]. Thirdly, our samples were recruited cross-sectionally, making it difficult to assess cause–effect relationships. Fourthly, allelic-association studies based on a single polymorphism in one candidate gene are subject to bias and confounding, and we cannot exclude the influence of some unmeasured factors in our patients.

Conclusions
In conclusion, ACE D/D homozygosis associates with a higher elevated TERalb and urine albumin levels, suggesting a common origin for the two phenomena. This abnormal systemic capillary permeability may accelerate atherosclerotic vascular disease, for example by retaining more atherogenic lipoproteins in the subendothelial space and/or preventing their egress; a morbid evolution to which D/D subjects may be more exposed [43]. However, this conceivable, but speculative, hypothesis needs to be tested prospectively.

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