The Deletion Polymorphism of the Angiotensin I-Converting Enzyme Gene Is Associated with Target Organ Damage in Essential Hypertension

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ABSTRACT

The activity of the renin-angiotensin-aldosterone system is thought to play a significant role in the development of target organ damage in essential hypertension. An insertion/deletion (I/D) polymorphism of the angiotensin I-converting enzyme (ACE) gene has recently been associated with increased risk for left ventricular hypertrophy and coronary heart disease in the general population. The D allele is associated with higher levels of circulating ACE and therefore may predispose to cardiovascular damage. The study presented here was performed to investigate the association between the ACE genotype, microalbuminuria, retinopathy, and left ventricular hypertrophy in 106 patients with essential hypertension. The ACE gene polymorphism was determined by polymerase chain reaction technique. Microalbuminuria was evaluated as albumin-to-creatinine ratio (A/C) in three nonconsecutive first morning urine samples (negative urine culture) after a 4-wk washout period. Microalbuminuria was defined as A/C between 2.38 to 19 (men) and 2.96 to 20 (women). Hypertensive retinopathy was evaluated by direct funduscopic examination (Keith-Wagener-Barker classification) and left ventricular hypertrophy by M-8 mode echocardiography. The distribution of the DD, ID, and II genotypes was 27, 50, and 23%, respectively. The prevalence of microalbuminuria, retinopathy, and left ventricular hypertrophy was 19, 74, and 72% respectively. There were no differences among the three genotypes for age, known duration of disease, body mass index, blood pressure, serum glucose, uric acid, and lipid profile. DD and ID genotypes were significantly associated with the presence of microalbuminuria (odds ratio, 8.51; 95% confidence interval, 1.07 to 67.85; P = 0.019), retinopathy (odds ratio, 5.19; 95% confidence interval, 1.71 to 15.75; P = 0.005) and left ventricular hypertrophy (odds ratio, 5.22; 95% confidence interval, 1.52 to 17.94; P = 0.016). Furthermore, patients with DD and ID genotypes showed higher levels of A/C (3.6 ± 0.9, DD; 2.6 ± 0.7, ID; 0.9 ± 0.2 mg/mmol, II; P = 0.0015 by analysis of variance) and increased left ventricular mass index (152 ± 4.7, DD+ID versus 133 ± 5.7 g/m², II; P = 0.01) compared with II patients. The D allele was significantly more frequent in patients with microalbuminuria (odds ratio, 2.59; 95% confidence interval, 1.24 to 5.41; P = 0.013) and in those with retinopathy (odds ratio, 2.44; 95% confidence interval, 1.21 to 4.90; P = 0.015). Multiple regression analyses performed among the entire cohort of patients demonstrated that ACE genotype significantly and independently influences the presence of retinopathy, left ventricular hypertrophy, and microalbuminuria. In conclusion, the D allele of the ACE gene is associated with microalbuminuria as well as with retinopathy and left ventricular hypertrophy, and seems to be an independent risk factor for target organ damage in essential hypertension.

Key Words: ACE gene polymorphism, renin-angiotensin-aldosterone system, left ventricular hypertrophy, retinopathy, microalbuminuria

Patients with essential hypertension are heterogeneous in their clinical characteristics and prognosis. Several factors besides the level of blood pressure values may contribute to the development of target organ damage and cardiovascular complications. Among these factors, an unambiguous role is played by the renin-angiotensin-aldosterone system (1).

The genes encoding components of the renin-angio-
tensin system are appealing candidates for a significant role in the development of cardiovascular damage in patients with hypertension. The renin-angiotensin-aldosterone system is present in circulating and tissue-based forms and is involved in sodium homeostasis, cardiovascular remodeling, and maintenance of vascular tone. Angiotensin I-converting enzyme (ACE) is a key component within the renin-angiotensin system, where it hydrolyzes angiotensin I to generate the powerful vasoconstrictor angiotensin II, and within the kallikrein-kinin system, where it inactivates the vasodilator bradykinin. In humans, the plasma level of ACE is genetically determined. The ACE gene, which has been mapped to human chromosome 17q23, has an insertion/deletion (I/D) polymorphism in intron 16. The mean plasma ACE level in DD subjects is about twice that of II subjects with ID subjects having intermediate levels (2,3). Although the I/D polymorphism of the ACE gene is considered to play a small role in the pathogenesis of hypertension, the higher levels of ACE associated with the D allele may lead to greater angiotensin II formation in cardiac and vascular tissues, predisposing a subject to cardiovascular damage.

In fact, the DD genotype has recently been reported to be a risk factor for left ventricular hypertrophy (4,5) and ischemic heart disease (6,7), both in the general population and in patients with diabetes mellitus (8), even though other studies could not confirm this association (9).

The study presented here was performed to explore the possibility that ACE gene polymorphism may be related to the development of target organ damage in essential hypertension.

METHODS

Study Population

Since January 1994, 106 consecutive patients (all Caucasian Europeans) with essential hypertension who were attending the outpatient clinic of our institution were asked to participate in this study, which was part of a larger clinical trial (M.A.G.I.C.: Microalbuminuria: A Genoa Investigation on Complications) approved by the Ethical Committee of our Institution. Exclusion criteria were the presence of neoplastic, hepatic and/or renal disease, chronic heart failure (New York Heart Association Classes III and IV), diabetes mellitus, severe obesity (defined as body weight >150% of ideal body weight), disabling diseases (such as dementia), or the inability of the patients to cooperate. Diagnosis of essential hypertension was made by the attending physician after complete medical history, physical examination, and routine biochemical analyses of blood and urine were obtained from every patient. Further investigation was carried out only when abnormalities were found in these analyses, or when other symptoms or signs suggesting secondary hypertension were present. Hypertension was defined according to Joint National Committee V criteria as an average blood pressure >140/90 mm Hg on at least three different occasions, or by the presence of antihypertensive treatment. None of the patients was on drug treatment at the time of the study. They had either never been treated for hypertension or had been taken off therapy at least 4 wk before the study. Twenty-four urinary collection was obtained in each patient for the measurement of creatinine and electrolytes. On the study day, height and weight were measured, then venous blood was drawn after an overnight fast in order to measure hematocrit, blood pressure was measured by a trained nurse on the right arm, with the patient in sitting position after a 5-min rest, with a mercury sphygmomanometer (cuff size 12.5 cm). The systolic and diastolic blood pressures were read to the nearest 2 mm Hg. Disappearance of Korotkoff's sounds (Phase V) was the criterion for diastolic blood pressure. The lowest of three consecutive readings were recorded. Body mass index (BMI) was calculated with the formula: BMI = weight (Kg)/height (m)². Standard electrocardiogram (12 leads) and funduscopic examination were obtained for each patient. Creatinine, BUN, electrolytes, uric acid, triglycerides, total and high-density lipoprotein cholesterol, and other standard chemistry evaluations were performed on serum and urine according to routine methods. Low-density lipoprotein cholesterol was calculated using Friedewald's formula (10). Family history and lifestyle habits were assessed by means of a standardized questionnaire.

Assessment of ACE Genotype

DNA was isolated from frozen whole blood containing EDTA as anticoagulant by a standard salting-out procedure, and was resuspended in a TE (10 mM Tris/1 mM EDTA, pH 7.6) buffer. Genomic DNA (approximately 0.25 μg) was amplified by polymerase chain reaction in a 50-μL reaction mixture containing 10 mM Tris-HCl, pH 8.8, 50 mM KCl, 1.5 mM MgCl₂, 0.1% Triton X-100 (Sigma Chemical Co., St. Louis, MO), 200 μM each dATP, dCTP, dGTP, dTTP, 0.12 μM each primer, and 0.5 U thermostable DNA polymerase (Dy-nazyme, Esco, Finland). The mixture was overlaid with mineral oil. In any given set of reactions, a negative control containing no genomic DNA and a positive control of known genotype were always included. The amplification was obtained after the following steps: 5 min of denaturation at 95°C, followed by 30 cycles of 1 min at 94°C, 1 min at 58°C, and 2 min of DNA synthesis at 72°C. First, all of the samples were analyzed using a pair of primers that amplify the entire intron 16, as already reported (11). To avoid ID/DD mistyping, a pair of primers that amplify a region inside intron 16 were also used to analyze all samples showing DD genotype as described (7). The samples were visualized after electrophoresis on a 2% agarose gel and ethidium bromide staining. Investigators were blinded as to type when genotyping was undertaken.

Microalbuminuria

The presence of microalbuminuria was evaluated, at the end of the wash-out period (if any) as the albumin-to-creatinine ratio (A/C) on three nonconsecutive, first morning urine samples. Only samples from patients with a negative urine culture were collected. Whenever a positive urine culture was found, urine samples were discarded, appropriate antibacterial treatment was instituted, and urine collections for albuminuria were repeated only after a second culture tested negative. The A/C was calculated as follows: urinary albumin concentration (milligram per liter)/urinary creatinine concentration (mmol per liter). Serum and urine creatinine levels were determined by the routine Jaffe's reaction. Urine albumin concentration was measured by a commercially available RIA kit (Sclavo, Cinisello Balsamo, Italy). An A/C between 2.38 and 19 (men) and between 2.96 and 20

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(women) was used to define microalbuminuria. These criteria proved to have good sensitivity and specificity in the detection of albumin excretion rate between 20 and 200 μg/min (12). The average of each patient's three A/C was used to indicate the level of albumin excretion.

Retinopathy

The presence, type, and extent of hypertensive retinopathy were investigated in a darkened room and under pupil dilatation. Direct ophthalmoscopy was carried out with a halogen ophthalmoscope: the first arteriovenous crossing at least one disc diameter from the disc in each quadrant was selected and assessed. Each quadrant of the fundus was photographed by means of a Zeiss fundus camera (Oberkochen, Germany) with a 30° field. Photographic slides were projected and evaluated by the same ophthalmologist, who was unaware of the patients' clinical data. Retinal features sought were venule nipping, venule deviation, and light reflex change at all clearly visualized crossings with comparably sized arterioles at least one disc diameter from the disc. The mean ratio of diameters of equivalent-order arterioles and venules in each quadrant was measured and the presence of focal arteriolar narrowing, hemorrhages, exudates, and papilledema was sought. Retinal lesions were classified according to Keith-Wagener-Barker classification (13).

Echocardiography

All echocardiographic studies were performed using an Acuson XP-128 ultrasound machine (Mountain View, CA). Echocardiograms were obtained at rest with patients supine in the left lateral position, by using standard parasentral and apical views. The overall monodimensional left ventricular measurements and the bidimensional (apical four and two chamber) views were obtained according to the recommendation of the American Society of Echocardiography (14,15). All tracings were obtained and read by a single observer who was blinded to the clinical characteristics of the patients under observation. Left ventricular mass was derived from the formula described by Devereux and associates (16):

\[
\text{LV mass (g)} = 0.80 \times 1.04 [(\text{VSTd} + \text{LVIDd} + \text{PWTd})^3 - (\text{LVIDd})^3] + 0.6
\]

where VSTd is ventricular septal thickness at end diastole, LVIDd is LV internal dimension at end diastole, and PWTd is LV posterior wall thickness at end diastole. Left ventricular mass was corrected for body surface area (LVMi), and expressed in units of grams/meter squared (g/m²). The presence of left ventricular hypertrophy (LVH) was defined for LVMi ≥134 g/m² (men) and ≥110 g/m² (women) (17). No patients showed dyssynergic areas that would invalidate the theoretical assumptions behind the cardiac mass calculations.

Statistical Analysis

All data are expressed as mean ± SE. Differences between variables were assessed using the appropriate statistical test based on the underlying distribution of the variables. One-way analysis of variance (ANOVA) with multiple comparison post-test were used to analyze data from patients with the three ACE genotypes. Either a nonparametric (Welch's t test) or a parametric (Student's t test) test was used to assess differences between patients with DD+ID and II genotypes. Differences between prevalences were assessed by chi-squared test or Fisher's exact test as appropriate. The calculation of odds ratios was performed to provide an estimate of the relative risk of microalbuminuria, retinopathy, and left ventricular hypertrophy in groups of patients with different genotypes. Piecewise linear regression analysis was performed to assess the contribution of ACE genotype and other variables on albumin excretion. This analysis may provide a better assessment of data when the nature of the relationship between one or more independent variables and a dependent variable changes over the range of the dependent variable. It also permits the identification of the point where discontinuity in the regression line occurs (i.e., the breakpoint of the regression) (18). Multiple regression analysis was performed to assess the independent contribution of ACE genotype (II = 0, ID+DD = 1) and other variables on the presence of retinopathy and left ventricular hypertrophy. A K-means cluster analysis was performed on the entire cohort of patients to identify different subgroups of patients according to the presence/absence of target organ damage and ACE D genotype (II = 0, DD+ID = 1). The appropriateness of the classification has been assessed by performing a standard between-group analysis of variance for each variable (19). All statistical analyses were performed using SAS (SAS Institute, Cary, NC) and Statistica (Statsoft Inc., Tulsa, OK) software.

RESULTS

The observed overall genotype distribution (DD, 27%; ID, 50%; and II 23%) was consistent with Hardy-Weinberg equilibrium and similar to previous reports in caucasian populations (20). There was an overall frequency of 54% for the D allele and 46% for the I allele, which compares closely with other white populations studied (21). The overall prevalence of microalbuminuria, retinopathy, and left ventricular hypertrophy were 19.74, and 72%, respectively. There were no differences in age, prevalence of family history for hypertension, current smokers, known duration of disease, BMI, blood pressure, serum glucose, uric acid, and lipid profile when patients were divided according to their genotype (Table 1).

Patients with DD and ID genotypes were significantly more likely to be microalbuminuric (DD, 37%; ID, 26%; II, 6%); chi-squared, 6.74, $P = 0.034$; chi-squared for trend, 6.50, $P = 0.01$; Table 1) and showed higher levels of A/C (DD, 3.6 ± 0.9; ID, 2.6 ± 0.7; II, 0.9 ± 0.2 mg/mmol; $P = 0.027$ by ANOVA; Table 1) compared with II patients. The odds ratio for the presence of microalbuminuria was 8.51 (95% confidence interval, 1.07 to 67.85; $P = 0.019$; Figure 1) in patients with DD and ID genotypes. The frequency of D allele was significantly higher in patients with microalbuminuria (0.69 versus 0.46; odds ratio, 2.59; 95% confidence interval, 1.24 to 5.41; $P = 0.013$; Table 2) compared with subjects who were normoalbuminuric. A piecewise linear regression analysis was performed among the entire cohort of patients to identify factors that independently influence A/C. A two-slope linear model was identified with a breakpoint value of 2.4, almost identical to the one predefined to indicate the passage from normo- to microalbuminuria. Age, BMI, LDL cholesterol, and ACE genotype significantly influence A/C levels and to-
### TABLE 1. Clinical characteristics of patients according to ACE gene polymorphism

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>DD</th>
<th>ID</th>
<th>II</th>
<th>P&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Patients</td>
<td>29</td>
<td>53</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Male/Female</td>
<td>18/11</td>
<td>28/25</td>
<td>15/9</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>43.6±2.2</td>
<td>49.7±1.3</td>
<td>47.0±1.9</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of Disease (months)</td>
<td>47.6±9.3</td>
<td>60.7±8.2</td>
<td>59.2±13.0</td>
<td>NS</td>
</tr>
<tr>
<td>Family History of Hypertension (%)</td>
<td>84</td>
<td>65</td>
<td>57</td>
<td>NS</td>
</tr>
<tr>
<td>Current Smokers (%)</td>
<td>52</td>
<td>38</td>
<td>40</td>
<td>NS</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>164±3</td>
<td>161±2.9</td>
<td>154±2.9</td>
<td>NS</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>106±1.3</td>
<td>103±1.0</td>
<td>102±1.1</td>
<td>NS</td>
</tr>
<tr>
<td>MBP (mm Hg)</td>
<td>125±1.6</td>
<td>122±1.4</td>
<td>119±1.5</td>
<td>NS</td>
</tr>
<tr>
<td>Body Mass Index&lt;sup&gt;c&lt;/sup&gt;</td>
<td>27.7±0.83</td>
<td>27.0±0.57</td>
<td>26.6±0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting Blood Glucose (mg/dL)</td>
<td>87.5±1.7</td>
<td>91.0±1.8</td>
<td>94.7±2.5</td>
<td>NS</td>
</tr>
<tr>
<td>Uric Acid (mg/dL)</td>
<td>5.3±0.3</td>
<td>5.4±0.2</td>
<td>5.3±0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>128±11</td>
<td>130±9</td>
<td>107±11</td>
<td>NS</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>220±10</td>
<td>212±6</td>
<td>214±7</td>
<td>NS</td>
</tr>
<tr>
<td>HDL Cholesterol (mg/dL)</td>
<td>46±3.7</td>
<td>49±2.0</td>
<td>50±2.7</td>
<td>NS</td>
</tr>
<tr>
<td>LDL Cholesterol (mg/dL)</td>
<td>154±9.9</td>
<td>133±5.2</td>
<td>143±6.5</td>
<td>NS</td>
</tr>
<tr>
<td>Urinary Na (mmol/day)</td>
<td>173±17</td>
<td>156±14</td>
<td>147±20</td>
<td>NS</td>
</tr>
<tr>
<td>A/C (mg/mmol)</td>
<td>3.6±0.9</td>
<td>2.6±0.7</td>
<td>0.9±0.2</td>
<td>0.027</td>
</tr>
<tr>
<td>Prevalence of Microalbuminuria (%)</td>
<td>37</td>
<td>26</td>
<td>6</td>
<td>0.034</td>
</tr>
<tr>
<td>Prevalence of Retinopathy (%)</td>
<td>83</td>
<td>80</td>
<td>44</td>
<td>0.009</td>
</tr>
<tr>
<td>Prevalence of LVH (%)</td>
<td>74</td>
<td>83</td>
<td>33</td>
<td>0.015</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data are mean ± SE; ACE, angiotensin-converting enzyme; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; AC, albumin/creatinine ratio; LVMI, left ventricular mass, corrected for body surface area; LVH, left ventricular hypertrophy; NS, not statistically significant.

<sup>b</sup> P values were calculated by one-way analysis of variance or chi-squared test as appropriate.

<sup>c</sup> Calculated by dividing the weight in kilograms by the square of the height in meters.

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Figure 1. Relative risk of microalbuminuria, retinopathy, and left ventricular hypertrophy in patients with essential hypertension and ACE DD and ID genotypes. Patients with ACE D genotypes (DD + ID) are more likely to have target organ damage than patients with ACE II genotype. LVH, left ventricular hypertrophy.

Together account for over 75% of variations in albuminuria (Table 3).

Hypertensive retinopathy was present in patients with DD and ID genotypes significantly more often (DD, 83%; ID, 80%; II, 44%; chi-squared, 9.47, P = 0.009; chi-squared for trend, 6.73, P = 0.009; Table 1) than in II patients. The odds ratio for the presence of hypertensive retinopathy was 5.19 in patients with DD and ID genotypes (95% confidence interval, 1.71 to 15.75, P = 0.005; Figure 1). The frequency of D allele was significantly higher in patients with hypertensive retinopathy (0.59 versus 0.37; odds ratio, 2.44; 95% confidence interval, 1.21 to 4.90, P = 0.015; Table 2).

Multiple regression analysis performed among the entire cohort of patients demonstrated that ACE genotype (P < 0.01), age (P < 0.01), and A/C (P < 0.02) significantly and independently influence the presence of retinopathy and together account for about 30% of variations in retinal status (r² = 0.292, Table 4).

DD and ID genotypes were associated with a significantly higher prevalence of left ventricular hypertrophy (DD, 74%; ID, 83%; II, 33%; chi-squared, 8.96, P = 0.01; Table 1). Patients with DD and ID genotypes showed increased LVMI (152 ± 5 versus 133 ± 6 g/m², P = 0.01, Table 1; odds ratio for the presence of left ventricular hypertrophy, 5.22; 95% confidence interval, 1.52 to 17.94, P = 0.016; Figure 1) compared with II patients. Multiple regression analysis demonstrated that ACE genotype (P < 0.03) and total cholesterol (P < 0.05) significantly and independently influence the presence of left ventricular hypertrophy.
TABLE 2. D-I allele frequency in essential hypertensive patients with and without target-organ damage

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Alb+</th>
<th>Alb-</th>
<th>Ret+</th>
<th>Ret-</th>
<th>LVH+</th>
<th>LVH-</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>0.69</td>
<td>0.46</td>
<td>0.59</td>
<td>0.37</td>
<td>0.60</td>
<td>0.50</td>
</tr>
<tr>
<td>I</td>
<td>0.31</td>
<td>0.54</td>
<td>0.41</td>
<td>0.63</td>
<td>0.40</td>
<td>0.50</td>
</tr>
<tr>
<td>Odds Ratio</td>
<td>2.59</td>
<td>2.44</td>
<td>1.52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% Confidence Interval</td>
<td>1.24 to 5.41</td>
<td>1.21 to 4.90</td>
<td>0.71 to 3.26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P Value</td>
<td>0.013</td>
<td>0.015</td>
<td>0.330</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Alb+ and Alb- indicate patients with and without microalbuminuria. Ret+ and Ret- indicate patients with and without retinopathy. LVH+ and LVH- indicate patients with and without left ventricular hypertrophy. The relative risk for organ damage in patients with D allele was calculated by Fisher's exact test.

TABLE 3. Significant determinants of microalbuminuria in essential hypertension (piecewise linear regression analysis)

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>Regression Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Below Breakpoint</td>
</tr>
<tr>
<td>Age</td>
<td>-0.0012</td>
</tr>
<tr>
<td>BMI</td>
<td>0.0239</td>
</tr>
<tr>
<td>LDL Chol</td>
<td>-0.0055</td>
</tr>
<tr>
<td>ACE</td>
<td>-0.1843</td>
</tr>
</tbody>
</table>

*Dependent variable: albuminuria (A/C); independent variables: Age, BMI, LDL Chol, ACE genotype. Breakpoint = 2.4 Regression degrees of freedom = 4-16, F = 3.66, P = 0.026. R² = 0.775. A/C indicates urinary albumin-to-creatinine ratio (mg/mmol); BMI, body mass index; LDL Chol, LDL cholesterol (mg/dL); ACE, angiotensin-converting enzyme genotype (I = 1, DD = 1, DD = 1).

TABLE 4. Multiple regression analysis of retinopathy

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>β</th>
<th>SEβ</th>
<th>Partial F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE</td>
<td>0.307</td>
<td>0.116</td>
<td>10.80</td>
<td>0.010</td>
</tr>
<tr>
<td>Age</td>
<td>0.296</td>
<td>0.111</td>
<td>8.63</td>
<td>0.010</td>
</tr>
<tr>
<td>A/C</td>
<td>0.268</td>
<td>0.116</td>
<td>7.98</td>
<td>0.024</td>
</tr>
</tbody>
</table>

*Dependent variable: retinopathy; independent variables = ACE genotype, Age, A/C. Regression degrees of freedom = 3-58, F = 7.977, P = 0.00015. Intercept = -0.247; R² = 0.292. ACE indicates angiotensin-converting enzyme genotype (I = 0, DD = 1); A/C, urinary albumin-to-creatinine ratio (mg/mmol).
and together account for about 25% of its variations (r² = 0.236, Table 5).

Patients with DD and ID genotypes showed a remarkably higher percentage of either at least two (odds ratio, 9.333; 95% confidence interval, 2.373 to 36.717, P = 0.0006; data not shown) or all three cardiovascular abnormalities investigated in this study (odds ratio, 15.9; 95% confidence interval, 0.9038 to 280.05, P = 0.007; data not shown). Furthermore, the D allele entails a threefold risk of having either at least two (odds ratio, 2.483; 95% confidence interval, 1.261 to 4.868, P < 0.011; data not shown) or all three of the above-mentioned abnormalities (odds ratio, 3.333; 95% confidence interval, 1.425 to 7.795, P = 0.006; data not shown). K-means cluster analysis of the entire cohort of patients allowed us to identify three subgroups that differ significantly (Table 6) for the presence (or absence) of retinopathy, microalbuminuria, left ventricular hypertrophy, and the frequency of DD or ID genotype (Figure 2), while showing no difference as far as other clinical and biochemical parameters are concerned. Patients in Cluster 2 (N = 15) show a lower than average frequency of ACE DD and ID genotypes as well as low prevalence of microalbuminuria and left ventricular hypertrophy. Patients in Cluster 1 (N = 31) show a frequency of DD and ID ACE genotypes similar to the average of the entire population and are characterized by a lower prevalence of microalbuminuria but higher prevalence of left ventricular hypertrophy. Finally, patients

TABLE 5. Multiple regression analysis of left ventricular hypertrophy

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>β</th>
<th>SEβ</th>
<th>Partial F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE</td>
<td>0.361</td>
<td>0.160</td>
<td>4.40</td>
<td>0.031</td>
</tr>
<tr>
<td>Chol</td>
<td>0.334</td>
<td>0.160</td>
<td>4.62</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*Dependent variable: left ventricular hypertrophy; independent variables: ACE genotype, Chol. Regression degrees of freedom = 2 to 30. F = 4.623, P = 0.178. Intercept = -0.290; R² = 0.236. ACE indicates angiotensin-converting enzyme genotype (I = 0, ID + DD = 1); Chol, serum total cholesterol (mg/dL).

TABLE 6. Multi-way, between-groups analysis of variance and contrast analysis of the prevalence of target-organ damage and ACE genotypes (I = 0, DD + ID = 1) in patients with essential hypertension divided into three clusters according to K-means cluster analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinopathy</td>
<td>2</td>
<td>3.944</td>
<td>0.025</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>2</td>
<td>4.414</td>
<td>0.016</td>
</tr>
<tr>
<td>ACE Genotype</td>
<td>2</td>
<td>4.330</td>
<td>0.017</td>
</tr>
<tr>
<td>Left Ventricular Hypertrophy</td>
<td>2</td>
<td>19.330</td>
<td>0.005</td>
</tr>
</tbody>
</table>

*The three clusters differ significantly for the presence/absence of target organ damage and ACE genotype. df, degrees of freedom.
Figure 2. Plot of frequency of target organ damage and ACE D genotype in patients with essential hypertension divided according to K-means cluster analysis. K-means cluster analysis was performed among the entire cohort of patients. Three different subgroups were identified according to the prevalence of target organ damage and the frequency of ACE genotype. Ref + indicates the presence of hypertensive retinopathy; Mi, microalbuminuria; ACE +, angiotensin-converting enzyme genotypes DD and ID; LVH, left ventricular hypertrophy. The three clusters differ significantly for each variable examined (see Table 6).

in Cluster 3 are characterized by a very high frequency of ACE DD and ID genotypes associated with a high prevalence of retinopathy, microalbuminurias, and left ventricular hypertrophy (Figure 2).

**DISCUSSION**

The principal finding of this study is that essential hypertensive patients with DD and ID genotypes of the ACE gene show a greater prevalence of target organ damage. The D allele seems to confer an increased risk for the development of microalbuminuria and retinopathy independently of blood pressure values as well as other commonly acknowledged cardiovascular risk factors, namely age, smoking habits, family history and known duration of hypertension, body mass index, lipid profile, uric acid, and fasting blood glucose. Although the DD genotype has been reported to be a risk factor for left ventricular hypertrophy (3,4) and ischemic and degenerative heart disease (22) in the general population, and for a more rapid progression of renal failure in nondiabetic renal disease (23,24), this is, to our knowledge, the first time that it has been reported in association with the development of multiple organ damage in essential hypertension. Our findings also support previous evidence that the renin-angiotensin-aldosterone system may play an important role in the pathophysiology of vascular and tissue damage in essential hypertension (1,25).

In the study presented here, the prevalence of microalbuminuria, retinopathy, and left ventricular hypertrophy was comparable with what has been reported in the literature (26-29).

Earlier studies have shown a high prevalence of retinopathy in patients with essential hypertension (27). Retinal changes are thought to reflect vascular and tissue damage and thereby the risk of future vascular events. In fact, hypertensive retinopathy has been previously reported to be an independent indicator of all-causes mortality (30). In the study presented here, patients with DD and ID genotypes had a fivefold greater risk for retinopathy (Figure 1) compared with II patients. The presence of the D allele entails a twofold greater risk for retinopathy (Table 2). Patients with Grades 1 and 2 lesions were analyzed together because of the substantial overlapping of the retinal features described (Table 1). However, when the severity of retinal changes was taken into consideration, a significant association with DD and ID genotypes was still present (chi-squared, 10.9, P = 0.028; data not shown). Furthermore, the frequency of D allele was higher in patients with more severe degrees of retinopathy (chi-squared, 10.3, P = 0.006; chi-squared for trend, 4.55, P = 0.033; data not shown). Multiple regression analyses demonstrated that the ACE polymorphism independently contributes to the presence of retinopathy and accounts for a significant part of variation in retinal status (Table 4).

Increased left ventricular mass is a well-known, powerful, independent predictor of cardiovascular morbidity and mortality in hypertension (31-33) and is thought to be a consequence of left ventricular pressure overload (34-36). The relatively high prevalence of left ventricular hypertrophy in our study (Table 1) is comparable to the one previously reported in the literature when similar sensitive and specific techniques were used (37,28). In the study presented here, patients with DD and ID genotypes had a higher probability of having left ventricular hypertrophy compared with II patients (Figure 1, Table 1). When analyzed together, DD and ID patients showed increased LVMI compared with II patients (151.9 ± 5 versus 133.2 ± 5 g/m², P = 0.01; data not shown). Multiple regression analyses demonstrated that ACE genotype is an independent predictor of the development of left ventricular hypertrophy and accounts for a significant part of variation in cardiac mass (Table 5). At present, data on the relationship between ACE gene and left ventricular hypertrophy are controversial. A recent study on a large group of subjects from the Framingham Heart Study failed to show a role of the ACE gene in influencing left ventricular mass (38). However, the results presented here are in accordance with previous studies reporting an association between the DD genotype of ACE and the risk of left ventricular hypertrophy in the general population including patients with hypertension (4,5). These contrasting results can be explained at least in part by the nonuniform genetic background of study populations and by the possible confounding effect of antihypertensive treatment.

Although hypertensive retinopathy and left ventricular hypertrophy have long been regarded as important complications of hypertension and predictors of cardiovascular death, the role of microalbuminuria as an important and independent cardiovascular risk factor or more likely as a marker itself of diffuse
endothelial vascular damage has only been acknowledged over the last few years. Several investigators have shown that microalbuminuria is a predictor of cardiovascular events in hypertensive patients (29,39). In the 10-yr Goteborg follow-up study (40), the predictive power of albuminuria exceeded that of total serum cholesterol and other cardiovascular risk factors. More recently, other authors have shown that in essential hypertension, microalbuminuria is associated with a higher degree of left ventricular hypertrophy (29,41) as well as hypertensive retinopathy (27). These data support the conclusion that microalbuminuria is a marker for early end-organ and cardiovascular damage in such patients. In the study presented here, patients with DD and ID genotypes not only showed higher levels of albuminuria but also a significantly higher prevalence of microalbuminuria compared with II patients (Table 1). DD and ID genotypes were associated with an 8.5 times greater probability of microalbuminuria compared with II patients (Figure 1). Piecwise linear regression analysis demonstrated that ACE genotype, together with age, BMI, and LDL cholesterol, strongly and independently influence variations in albumin excretion (Table 3). Interestingly, all these independent variables are positively related to A/C only above the breakpoint of the regression line, thus indicating that their contribution to variations in albumin excretion changes and is more evident in patients with persistent microalbuminuria. When taken together, these data strongly suggest a role for ACE gene polymorphism as a potential genetic risk factor for cardiovascular damage.

Several reasons make the ACE gene, among the renin-angiotensin genes, one of the major etiological candidates for the development of cardiovascular injury. First, the gene is highly expressed in endothelial cells and in the heart. Second, the levels of ACE in plasma and inside the cells show quantitative variations related to gene polymorphism. An increased enzyme concentration, associated with the DD and ID genotypes, may lead to an increase in AngII generation and bradykinin degradation. Bradykinin acts on endothelial beta-kin receptors and is able to stimulate nitric oxide formation by endothelial nitric oxide-synthetase and prostacyclin synthesis. Furthermore, experimental studies suggest that AngII may stimulate cardiac protein synthesis (42-44) whereas bradykinin may have an antiproliferative effect (45,46). Moreover, I/D ACE polymorphism was shown to modulate the intracellular level of ACE in T lymphocytes (47). In addition, recent studies have demonstrated elevated cardiac expression of ACE messenger RNA and ACE activity in experimental animals with pressure-overload left ventricular hypertrophy (46,48) and in patients with heart failure (49). Therefore it is tempting to speculate that, in patients with DD genotype, higher ACE activity in cardiac and vascular tissues might facilitate cellular hypertrophy, extracellular matrix formation (50,51), and sympathetic neurotransmission (52). All of the above might contribute to the development of vascular and tissue damage in essential hypertension. The results of the study presented here are in accordance with previous observations, suggesting that increased activity of the renin-angiotensin-aldosterone system might play a role in the development of target organ damage in essential hypertension. Erley et al. (53) demonstrated the presence of altered renal hemodynamics and higher prevalence of left ventricular hypertrophy, retinopathy, and microalbuminuria in young essential hypertensive patients with hyperresponsivity of the renin-angiotensin-aldosterone system. Accordingly, Alderman et al. (25) showed that plasma renin activity is important and independent risk factor for cardiovascular complications in a large number of patients with essential hypertension, prospectively followed-up for several years. The fact that in our study the association of microalbuminuria, retinopathy, and left ventricular hypertrophy was found far more often in patients with the ACE D allele is intriguing, because these three different expressions of early organ damage may be regarded as the end point of pathophysiological processes, promoted at least in part by increased angiotensin II levels. This conclusion seems to be supported by results of K-means cluster analysis, which identifies three distinct subgroups differing significantly in both the frequency of ACE genotype and in the prevalence of organ damage. Patients with a high frequency of DD and ID ACE genotypes (Cluster 3, Figure 2 and Table 6) show a remarkably higher prevalence of retinopathy, microalbuminuria, and left ventricular hypertrophy, whereas those with a low frequency of the D allele (Cluster 2) seem to be protected from developing organ damage.

Finally, salt intake has been shown to be related to both left ventricular hypertrophy and microalbuminuria in hypertensive patients (54). In the study presented here, there was no difference in urinary Na excretion among the three groups of patients according to ACE genotypes (Table 1). However, the study design does not allow us to exclude a contribution of dietary salt intake in the development of target organ damage.

Although the data presented need to be interpreted with some caution because of the limited number of patients studied, both the homogeneity of the genetic background (all patients were of Caucasian origin) and the frequency of different ACE genotypes, which was almost identical to the one previously reported in larger studies on Caucasian patients, led us to believe that our patients are representative of a larger population with essential hypertension.

In conclusion, the results of this study suggest that the deletion polymorphism of the ACE gene might be an independent risk factor for the development of organ damage in hypertensive patients. From a clinical perspective, this implies the possibility of early identification of patients at increased risk for development of target organ damage, by determining ACE polymorphism. Therefore, a greater effort can be made
to prevent cardiovascular complications and to treat hypertension and other modifiable risk factors in these patients. Whatever the underlying molecular mechanisms linking the ACE-gene polymorphism to the development of target organ damage may be, the determination of the ACE genotype could be helpful in the assessment of cardiovascular risk and in planning treatment strategies in patients with essential hypertension. Large longitudinal trials are needed to actually determine the incidence of target organ damage and cardiovascular complications in patients with high-risk genotypes and to clarify whether antihypertensive agents that directly exert their action on the renin-angiotensin system may provide additional organ protection beyond blood pressure control.

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REFERENCES
