Exclusion of the ACE D/I Gene Polymorphism as a Determinant of Endothelial Dysfunction

Gian Paolo Rossi, Stefano Taddei, Agostino Virdis, Lorenzo Ghiadoni, Giovanna Albertin, Stefania Favilla, Isabella Sudano, Achille C. Pessina, Antonio Salvetti

Abstract—A deletion/insertion (D/I) polymorphism within the ACE gene may increase the risk of cardiovascular events through still unknown mechanisms. The latter may involve increased angiotensin II–induced NO breakdown and/or reduced agonist-mediated NO release. We therefore investigated whether the D allele of the ACE gene affects endothelium-dependent vasodilatation in mild-to-moderate primary hypertensive patients and healthy normotensive subjects. We compared in a cross-sectional study the forearm blood flow response of the 3 D/I genotypes with 5 incrementally increasing doses of the endothelium-dependent vasodilator acetylcholine (0.15, 0.45, 1.5, 4.5, and 15 μg · 100 mL⁻¹ · min⁻¹) in 142 subjects: 103 mild-to-moderate uncomplicated primary hypertensives (49.3±9.1 years old, 152±11/99±5 mm Hg) and 39 normotensives (44.6±15.3 years old, 122±12/78±6 mm Hg). We also assessed the endothelium-independent vasodilatation in the forearm, as blood flow response to 3 incrementally increasing doses of sodium nitroprusside (1, 2, and 4 μg · 100 mL⁻¹ · min⁻¹). The overall genotype distribution was II, n=10; ID, n=70; and DD, n=62. It did not differ significantly between primary hypertensives and normotensives. A significant blunting of endothelium-dependent vasodilatation in primary hypertensive patients compared with normotensive subjects (P<0.001) was found. No effect of the DI genotype on endothelium-dependent and -independent vasodilatation was detected. Thus, these results obtained in a relatively large population do not support the contention that the D allele is associated with a blunting of either stimulated endothelial NO or donated NO responses in both mild-to-moderate primary hypertensive patients and normotensive subjects. (Hypertension. 2001;37:293-300.)

Key Words: hypertension, arterial gene expression kininase II nitric oxide vasodilatation

Primary (essential) arterial hypertension is a highly heterogeneous genetically complex disease. About one third of blood pressure (BP) variance has been attributed to genetic factors, and therefore polymorphisms of both candidate genes of primary hypertension and anonymous markers have been aggressively investigated, albeit with controversial results (for a review, see Lindpaintner1). By allowing stratification of patients into pathophysiologically homogeneous subsets, the identification of intermediate phenotypes of primary hypertension might greatly enhance the power of studies that investigate the genetic determinants of primary hypertension and thus be an important step in furthering our understanding of the basis of this common condition.

Angiotensin (Ang) I–converting enzyme (ACE, kininase II) is a zinc membrane–bound metallopeptidase that governs the conversion of Ang I to Ang II and the degradation of bradykinin at the endothelial surface.2,3 In 1990, a deletion/insertion (D/I) polymorphism within the ACE gene was identified and shown to account for about half of the variance of serum ACE concentrations in the normal population.4 The relevance of this polymorphism for human cardiovascular disease (CVD) remained uncertain until it was reported that homozygosity for the D allele was associated with both an increased risk and a parental history of fatal myocardial infarction compared with that for the I allele.5,6 The D allele has thereafter been associated with other CVD, including dilated and ischemic cardiomyopathy, coronary and carotid artery disease, coronary artery spasm, restenosis, left ventricular hypertrophy in hypertensives, left ventricular dysfunction, and, more recently, atherosclerotic renovascular hypertension.7–14 However, opposite results for almost every clinical association have also been published, and therefore the value of the D/I genotyping for the purpose of cardiovascular risk stratification has been challenged15–23 (for reviews, see Butler et al24 and Agerholm-Larsen et al25). Furthermore, the mechanisms by which the D allele would lead to a generalized increase in CVD risk remain largely speculative.

Compelling evidence indicates that endothelial dysfunction, defined as an impaired endothelium-dependent vasorelaxation, precedes macrovascular disease in most human conditions associated with atherosclerosis, such as primary hypertension, cigarette smoking, diabetes mellitus, hypercho-

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lesterolemia, and aging. Because endothelial dysfunction was also observed in the normotensive offspring of hypertensive parents, it was suggested to be genetically determined. Thus, endothelial dysfunction could not only be a hallmark of conditions that carry an excess risk of CVD and also represent an early-intermediate phenotype of arterial hypertension.

Among several candidate genes of endothelial dysfunction, the ACE gene appears to be a likely one because (1) it is anchored via its carboxyl terminus to the endoluminal side of endothelial cell plasma membrane, from which it can be released in the bloodstream and (2) the increased plasma ACE activity found in subjects with the D allele could decrease bradykinin bioactivity with ensuing blunting of receptor-mediated release of NO. Furthermore, even though the literature is variable on whether Ang II effects are enhanced Ang II production can increase levels of superoxide through increased activity of NADH/NADPH oxidase activity and thus lower the bioactivity of NO. In essential hypertension, oxidative stress-induced reduction in NO availability has been associated to impaired endothelium-dependent vasodilation. To our knowledge, the ACE gene has been investigated thus far in relation to endothelial dysfunction in only few studies of either normotensive or a small number of hypertensive patients with conflicting results.

Thus, within a large prospective collaborative project with the aim of identification of the genes of endothelial dysfunction, we investigated (1) whether the D/I polymorphism could be associated to impaired endothelium-dependent vasodilation in essential hypertensive patients and (2) whether this potential effect would differ between primary hypertensive patients and normotensive subjects.

**Methods**

**Study Design**

One hundred three primary hypertensive patients and 39 healthy normotensive volunteers participated in the present study, which was approved by the Medical Ethics Committee of the universities of Pisa and Padova. Subjects with hypercholesterolemia (total cholesterol >5.2 mmol/L), diabetes mellitus, cardiac or/cerebral ischemic vascular disease, impaired renal function, and other major pathologies were excluded from the study. In accordance with institutional guidelines, all patients were aware of the investigational nature of the study and gave written consent.

Subjects were defined as normotensive according to the absence of family history of essential hypertension and BP values of <140/90 mm Hg. Normotensive subjects were recruited among the individuals afferent to our department (staff, relatives of patients) provided they had demographic characteristics comparable to those of hypertensive patients. Primary hypertensive patients were recruited from among the newly diagnosed patients in the outpatient clinic of the Department of Internal Medicine of the University of Pisa if they reported a positive family history of essential hypertension, whenever supine arterial BP (after 10 minutes of rest) measured with mercury sphygmomanometry (with phase V Korotkoff), 3 times at 1-week intervals for 1 month, was consistently found to be >140/90 mm Hg. Secondary forms of hypertension were excluded through routine diagnostic procedures. Patients were enrolled if they had never been treated (n=74) or had spontaneously discontinued their pharmacological treatment (n=29). Any other pharmacological treatment was discontinued for ≥2 weeks before the study was performed.

Subjects were defined as a smoker or an ex-smoker if they smoked >5 cigarettes/d or if they had refrained from smoking for the previous 5 years, respectively.

The selection was made prospectively on a consecutive basis. Because these inclusion criteria were the same as those required for studies on endothelial function in essential hypertension, some normotensive control subjects and essential hypertensive patients had also been enrolled in other smaller ongoing studies that investigated mechanistic aspects of endothelial dysfunction; one of the studies has been published.

**Experimental Procedures**

Endothelial function was assessed with the perfused forearm technique. Briefly, the brachial artery was cannulated for drug infusion at systemically ineffective rates, intra-arterial BP measurement, and heart rate monitoring. Forearm blood flow (FBF) was measured in both forearms (experimental and contralateral forearm) with strain-gauge venous plethysmography. Circulation to the hand was occluded 1 minute before FBF measurement by inflation of a pediatric cuff around the wrist at suprasystolic BP. Forearm volume was measured according to the water displacement method. Details concerning the method as performed in our laboratory, including sensitivity and reproducibility, have already been published.

Endothelium-dependent vasodilatation was estimated by performing a dose–response curve to intra-arterial acetylcholine (ACH) (cumulative increase of the infusion rates 0.15, 0.45, 1.5, 4.5, and 15 μg·100 mL forearm tissue−1·min−1 for 5 minutes at each dose), whereas endothelium-independent vasodilatation was assessed with a dose–response curve to intra-arterial sodium nitroprusside (SNP), a direct smooth muscle cell relaxant compound (cumulative increase by 1, 2, and 4 μg·100 mL forearm tissue−1·min−1 for 5 minutes at each dose). These rates were selected to induce vasodilatation comparable to that obtained with ACh. The ACh or SNP infusion sequence was randomized; 30-minute washout was allowed between each dose–response curve. These procedures were carried out in the Department of Internal Medicine of the University of Pisa.

**Extraction of DNA and ACE Genotyping**

The blood was collected in EDTA and stored at −20°C. DNA was extracted from whole blood according to standard procedures. The quantity of DNA was confirmed with spectrophotometry. Because misotyping of heterozygous has been reported, genotypes for the ACE D/I polymorphism were determined in the presence of 5% (vol/vol) DMSO. Heterozygosities were verified with further polymerase chain amplification with a 3′ primer (5′-CCC GCC ACT ACG CCC GGC TAA TT-3′) specific of the insertion, as described previously. All of these procedures were carried out in the Department of Clinical and Experimental Medicine of the University of Padua.

**Statistical Analysis**

Results are expressed as mean±SD; SEM values were used in the figures for visual clarity. FBF measurements were compared between genotypes with a generalized linear model for repeated measures ANOVA. Raw (unadjusted) FBF values were at first compared between primary hypertensive patients and normotensive subjects and between D/I genotypes. Age, serum glucose, and total plasma cholesterol values were then entered as covariates in the model and used to adjust the FBF response to ACh and SNP. For both raw and adjusted data analyses, it was decided a priori to use a multivariate test to detect significant effects because it does not require the sphericity assumption. For multiple comparison between genotypes, the Holm’s sequential Bonferroni procedure was used to locate the origin for any significant difference. The ACE genotype effect was tested both in normotensive subjects and in hypertensive patients separately and in the whole population. Terms for interaction for diagnostic strata and for genotype-diagnostic category were also included in the models. A value of P<0.05 was considered statistically significant. All analyses were carried out with the SPSS for Windows statistical package (version 9.0; SPSS Inc).
TABLE 1. Demographic and Clinical Characteristics of Primary Hypertensive Patients and Normotensive Control Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Primary Hypertensive Patients (n=103)</th>
<th>Normotensive Control Subjects (n=39)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td></td>
<td>49.3±9.1</td>
<td>44.6±15.3</td>
<td>0.029</td>
</tr>
<tr>
<td>Gender, M/F, n</td>
<td></td>
<td>89/14</td>
<td>21/18</td>
<td>NS</td>
</tr>
<tr>
<td>Nonsmoker/smoker/ex-smoker, n</td>
<td></td>
<td>73/18/12</td>
<td>35/2/2</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td></td>
<td>26.7±2.7</td>
<td>23.4±3.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum creatinine, µmol/L</td>
<td></td>
<td>90.9±13.6</td>
<td>84.5±15.5</td>
<td>0.018</td>
</tr>
<tr>
<td>Serum uric acid, mmol/L</td>
<td></td>
<td>0.32±0.07</td>
<td>0.28±0.065</td>
<td>0.004</td>
</tr>
<tr>
<td>Serum Na⁺, mmol/L</td>
<td></td>
<td>142±2.6</td>
<td>141±1.8</td>
<td>NS</td>
</tr>
<tr>
<td>Serum K⁺, mmol/L</td>
<td></td>
<td>3.9±0.4</td>
<td>4.1±0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Supine PRA, ng Ang I · mL⁻¹ · h⁻¹</td>
<td></td>
<td>1.39 (0.21–0.97)</td>
<td>0.80 (0.17–0.38)</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma aldosterone, pmol/L</td>
<td></td>
<td>23.9 (3.14–17.5)</td>
<td>18.5 (2.14–11.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Na⁺ urinary excretion, mmol/24 h</td>
<td></td>
<td>117±82</td>
<td>138±81</td>
<td>NS</td>
</tr>
<tr>
<td>K⁺ urinary excretion, mmol/24 h</td>
<td></td>
<td>50±24</td>
<td>44±16</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td></td>
<td>67±5</td>
<td>65±4</td>
<td>0.012</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td></td>
<td>152±11</td>
<td>122±12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td></td>
<td>99±5</td>
<td>78±6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean BP, mm Hg</td>
<td></td>
<td>117±6</td>
<td>93±7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glycemia, mmol/L</td>
<td></td>
<td>5.17±0.6</td>
<td>4.89±0.7</td>
<td>0.018</td>
</tr>
<tr>
<td>LVMI, g/m² BSA</td>
<td></td>
<td>119±19</td>
<td>95±11</td>
<td>0.001</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td></td>
<td>216±32</td>
<td>194±35</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td></td>
<td>48±12</td>
<td>56±13</td>
<td>0.002</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td></td>
<td>135±44</td>
<td>109±48</td>
<td>0.003</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td></td>
<td>135±64</td>
<td>100±58</td>
<td>0.006</td>
</tr>
</tbody>
</table>

PRA indicates plasma renin activity; Ang, angiotensin; LVMI, left ventricular mass index. Values are mean±SD, except for PRA, which is expressed as median and range, and for LVMI, which is expressed as mean±SEM.

Results

Demographic Characteristics and ACE Genotype Distribution

The demographic and clinical characteristics of the primary hypertensive patients and normotensive subjects are shown in Table 1. Collectively, 84% were nonsmokers and ex-smokers and 16% were current cigarette smokers; the latter reported to smoke <5 cigarettes/d. Besides the obvious differences of BP and left ventricular mass index, the primary hypertensive patients were ≈5 years older and had significantly higher body mass index, serum creatinine, uric acid, glucose, triglycerides, and total cholesterol and lower HDL cholesterol values compared with the normotensive subjects. The distribution of the different ACE genotypes was II, n=10 (7%); DI, n=70 (49.3%); and DD, n=62 (43.7%), corresponding to an overall proportion of the D and I allele of 0.69 and 0.32, respectively. There was no deviation of the observed from the expected overall genotype distribution in both primary hypertensive patients and normotensive subjects or from the Hardy-Weinberg equilibrium. The demographic and clinical features of our population divided by DI/ genotype are shown in Table 2. There were no significant differences between genotypes in any of the variables examined.

Baseline FBF

There were no significant differences in baseline FBF (mean±SD) between primary hypertensive patients (3.18±0.60 mL · min⁻¹ · 100 mL⁻¹) and normotensive subjects (3.11±0.58) and between genotypes (II 3.11±0.46, ID 3.16±0.61, DD 3.14±0.60) on the day of the ACh study. Similarly, there were no significant differences in baseline FBF between primary hypertensive patients (3.30±0.57) and normotensive subjects (3.16±0.52) and between different genotypes (II 3.10±0.49, ID 3.30±0.64, DD 3.24±0.52) on the day of the SNP study (Figure 1).

Endothelium-Dependent Vasodilatation

ACh induced a significant increase of FBF in both primary hypertensive patients and normotensive subjects. This increase was significantly blunted in the former, compared with the latter, starting from the 1.5 µg · 100 mL forearm tissue⁻¹ · min⁻¹ dosage of ACh (not shown). At the maximum dosage of ACh, the FBF was 16.80±4.9 versus 20.17±6.4 mL · min⁻¹ · 100 mL⁻¹ (P<0.005) in primary hypertensive patients and normotensive subjects, respectively. The blunted vasodilatory response in primary hypertensive patients compared with normotensive subjects was evident when both unadjusted and adjusted (for age, serum glucose, and total serum cholesterol) data were
examined. In the whole population, we found a significant effect (P<0.0001) of age, but not of D/I genotype, on FBF response, when either unadjusted or adjusted data were considered (Table 3). Similarly, no difference of endothelium-dependent vasodilatation between genotypes was evident when primary hypertensive patients and normotensive subjects were analyzed separately or by 2-way repeated measures ANOVA.

**Endothelium-Independent Vasodilatation**

SNP significantly increased FBF in both primary hypertensive patients and normotensive subjects. There was no difference between the former and the latter at any dosages of SNP, including the maximum, when both unadjusted (16.65±6.13 versus 18.96±4.99, NS) and adjusted data were examined (not shown). A significant effect of the I allele on endothelium-independent vasodilatation was detected when unadjusted data were used (Figure 1B) with subjects homozygous for the I allele exhibiting a significantly lower increase of FBF in response to SNP, compared with both of the other genotype subjects (Figure 1). However, this effect was no longer seen when adjusted data were taken into consideration (Table 4).

**Discussion**

Compelling evidence indicates that the D allele of the ACE gene is related to increased plasma levels of ACE. In contrast, the data that support the contention that the D allele could also be a general marker of CVD are controversial. Positive studies have in fact been contradicted not only by negative results basically for all types of CVD (for reviews, see Koch et al and Samani et al) but also by a study showing an association of the D allele with longevity in centenarians.

The mechanisms responsible for the supposed predisposition to developing CVD also remain largely speculative, because the D/I polymorphism is located in intron 16 (ie,
TABLE 4. Results of an Among-Subjects Multivariate Test for the FBF Response to SNP

<table>
<thead>
<tr>
<th>Source</th>
<th>Mean of Squares</th>
<th>F</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>134.494</td>
<td>3.612</td>
<td>0.061</td>
</tr>
<tr>
<td>D/I genotype</td>
<td>23.971</td>
<td>0.644</td>
<td>0.528</td>
</tr>
<tr>
<td>Age</td>
<td>2.489</td>
<td>0.067</td>
<td>0.797</td>
</tr>
<tr>
<td>Mean BP</td>
<td>117.884</td>
<td>3.166</td>
<td>0.079</td>
</tr>
<tr>
<td>Glycemia</td>
<td>45.182</td>
<td>1.214</td>
<td>0.274</td>
</tr>
<tr>
<td>Total serum cholesterol</td>
<td>2.431</td>
<td>0.065</td>
<td>0.799</td>
</tr>
<tr>
<td>D/I genotype<em>age</em>mean</td>
<td>22.918</td>
<td>0.616</td>
<td>0.607</td>
</tr>
<tr>
<td>BP<em>glycemia</em>total serum choles</td>
<td>37.232</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A, Changes in FBF in response to an intrabrachial artery infusion of the endothelium-dependent vasodilator Acetylcholine in the subjects divided according to D/I ACE genotype. No significant effect of the D/I ACE genotype on the increase of FBF was observed, both when the raw (unadjusted) and when the adjusted (for the effect of age, serum glucose levels and total serum cholesterol) data (not shown) were examined. B, Changes in FBF in response to an intrabrachial artery infusion of the endothelium-independent vasodilator sodium nitroprusside (SNP) in the subjects divided according to D/I ACE genotype. Subjects homozygous for the I allele showed a significantly lower FBF increase in response to SNP compared with DI heterozygous when raw data were examined. However, this difference was no longer evident when FBF changes were adjusted for the effect of age, serum glucose levels, and total serum cholesterol (not shown). Data are mean±SEM.

Recent data have, however, been published that suggest the D allele could be a cause or a marker of impaired endothelium-dependent vasodilatation, thereby providing a clue to the potential mechanisms accounting for the increased risk of CVD.37,38

To further address this possibility, we used venous occlusion plethysmography in a relatively large population of mild-to-moderate primary hypertensive patients, most of whom had never been previously treated, and in a group of healthy normotensive subjects. All underwent careful genotyping for the ACE D/I polymorphism and determination of the FBF response to the local administration of the endothelium-dependent vasodilator ACh and of the endothelium-independent vasodilator SNP. We confirmed that the response to ACh was significantly blunted in the primary hypertensive patients compared with normotensive subjects, in keeping with previous results.49–51 However, we could not find any effect of the D/I genotype on endothelium-dependent vasodilatation (Figure 1A) both when the effect of the ACE genotype was tested in the population of normotensive and hypertensive as a whole and when diagnostic categories were assessed separately.

We did observe significant differences between different D/I ACE genotypes in the raw FBF response to SNP. Subjects who harbor the D allele had significantly enhanced endothelium-independent vasodilatation compared with those who were homozygous for the I allele (Figure 1B), a finding that suggests that this polymorphism can influence the arteriolar structural remodeling process and/or cGMP function. It is crucial to emphasize, however, that in this study of consecutive cases, there were significant differences in age, serum glucose levels, and serum lipid profile between primary hypertensive patients and normotensive subjects (Table 1). Accordingly, it was conceivable that these differences might have led to a failure to detect significant differences of FBF responses between genotypes. Therefore, we used the alternative approach of examining the endothelium-dependent and -independent vasodilatation responses with a multivariate technique that enabled adjustment of FBF changes for the factors that are known to affect the FBF responses (ie, aging, serum glucose, and total serum cholesterol). After this adjustment, whereas the FBF response to ACh remained significantly blunted in primary hypertensive patients compared with normotensive subjects, no significant effect of the D/I ACE genotype on the FBF response to ACh emerged. The significant association of D allele with endothelium-independent vasodilatation was no longer significant when adjusted data were examined, suggesting that it was a spurious finding likely due to the effect of confounding variables.

The present results disagree with those of previous studies that investigated the relationship between FBF response to both ACh and SNP and D/I polymorphism.33,34 According to Perticone et al,37 who studied a smaller population of never-treated primary hypertensive patients (n=32) and normotensive control subjects (n=24), the DD genotype would be associated with significant blunting of endothelium-dependent vasodilatation but not of endothelium-independent vasodilatation. More recently, a blunted endothelium-dependent vasodilatation was reported in healthy young normotensive university students (n=68) carrying the D allele compared with II homozygous subjects.34 However, in this latter study, a blunted endothelium-independent vasodilatation was also found in DD homozygous subjects, thereby suggesting that the blunted endothelium-dependent vasodilatation could be in part accounted for by either a dysfunctional cGMP pathway or enhanced arteriolar structural changes, or both.34 Although collectively these findings would be consistent with the hypothesis that the D allele might increase the
risk of developing CVD, they conflict not only with our present results but also with those of other studies, particularly those with the largest sample size and thus the highest statistical power. Consistent with our data, Steeds et al. found no effect of the D/I genotype on the vascular responses in vitro to both Ang II and Ang I in resistance arteries from patients undergoing colonic resection for cancer. Similarly, Celermajer et al. also found no differences between D/I genotypes in the in vivo brachial artery responses of 184 normotensive nondiabetic lifelong nonsmokers using flow-mediated dilatation.

A first possible explanation for these differences could reside in the different criteria used for subject enrollment. In this study, we did our best to exclude previously heavy cigarette smokers, because it has been contended that the ACE genotype and smoking produce additive detrimental effects on endothelial function, although the blunting of endothelial function by smoking was not dose-dependently related to the D allele. Accordingly, the present results might not apply to populations of primary hypertensive patients and normotensive subjects that include a large proportion of smokers.

Nevertheless, the divergence between the present and previous results seems difficult to reconcile. Our population of both primary hypertensive patients and normotensive subjects had a distribution of the D and I allele of 0.69 and 0.31, respectively, which is quite similar to those previously reported in studies of white individuals. At variance, Struthers et al. reported the D allele to be less prevalent than the I allele (0.40 and 0.60, respectively) in their sample of young normotensive students, and therefore a selection bias was likely in this latter study, as the authors acknowledged.

Another crucial difference concerns the results with SNP. The present finding of an increased endothelium-independent vasodilatation in subjects harboring the D allele is opposite to that previously reported in normotensive subjects, but consistent with previous reports of an association of the I allele with essential hypertension and insulin resistance. However, it is important to emphasize that this association was no longer significant after proper adjustment of FBF responses for the effects of age, serum glucose, and total plasma cholesterol, clearly indicating the crucial importance of control for the effect of confounding variables.

Finally, negative results should always raise the question on whether the power of a study was adequate to avoid a type II (β) statistical error. In this regard, we point out that compared with previous studies that yield positive results, our study has a sample size more than twice as large and therefore a much higher power to detect an effect of the D/I genotype. Furthermore, we used higher ACh and SNP infusion rates, which induced a degree of vasodilatation to ACh of 6- and 5-fold above baseline in normotensive subjects and hypertensive patients, respectively, and a degree of vasodilatation to SNP of 5-fold above baseline in both study populations. Because these ranges of vasodilatation are higher than those attained in previous studies, we should have been able to achieve a much better discrimination of FBF responses between genotypes and therefore to detect any D/I genotype–related effect, if biologically real.

Formal calculations of statistical power showed that by assuming a 2-tailed α of 0.05 and the observed spread of FBF values, our study had a 95% power to detect a difference of 5.5 and 8.3 mL·min⁻¹·100 mL⁻¹ in the maximal FBF responses to ACh and SNP, respectively, between DD and II genotypes.

Of further interest, 78 various sites, of which 17 are in absolute linkage disequilibrium with the Alu D/I polymorphism and thus likely to give similar results in association studies, were recently identified in the ACE gene. These molecular variants resolved into 13 distinct haplotypes, which have not been considered thus far in studies that looked at associations with cardiovascular diseases. Furthermore, a major genetic subdivision in the deletion clade in European Americans, which could enable a more detailed analysis of cardiovascular phenotypes, has been identified. It is therefore likely that these novel information coupled to a wider application of high-density DNA-probe microarrays technology to achieve a more accurate molecular characterization of ACE gene variants can improve substantially our understanding of the impact of variations of this gene on cardiovascular disease in the near future.

Conclusions

By showing that the D allele of the ACE gene is associated neither with impaired NO pathways nor with the blunted endothelium-dependent vasodilatation observed in primary hypertensive patients compared with normotensive subjects, these data provide no support to the contention that this gene polymorphism has an impact in terms of NO-mediated endothelial pathway. Interestingly, we found an association of the I allele with a blunted endothelium-independent vasodilatation response when raw FBF were examined, suggesting the possibility of enhanced structural changes of the skeletal muscle microcirculation associated with this allele in humans and/or a dysfunctional cGMP pathway. However, this association was no longer seen after adjustment for the confounding effect of covariates, such as age, serum glucose, and total cholesterol levels, that are known to have an influence on FBF responses. This observation, while underscoring the need of proper adjustment of the data, indicates that extreme caution should be advised in interpretation of the results of cross-sectional studies carried out on small series of primary hypertensive patients and normotensive subjects. The complexity of allelic variation within the ACE gene, the number of variants identified, and their linkage disequilibrium further emphasize the importance of the cautious interpretation of association studies with a single polymorphic site.

Acknowledgments

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