ACE Genotype and Endothelium-Dependent Vasodilation of Conduit Arteries and Forearm Microcirculation in Humans

Guido Arcaro, Anna Solini, Tiziano Monauni, Anna Cretti, Barbara Brunato, Alessandro Lechi, Renato Fellin, Marco Caputo, Claudio Cocco, Enzo Bonora, Michele Muggeo, Riccardo C. Bonadonna

Abstract—The ACE gene is a candidate gene for cardiovascular disease. Endothelial dysfunction is considered an intermediate phenotype in the pathogenesis of hypertension and atherosclerosis. We evaluated the role of ACE gene polymorphism in endothelial function of young healthy humans. We assessed ACE genotype (deletion [D]/insertion [I] polymorphism) in 92 young healthy individuals. In 88 of them, endothelium-dependent (flow-mediated) vasodilation and endothelium-independent (nitroglycerin-induced) vasodilation were measured in the common femoral artery and in the brachial (n=84) artery by echo Doppler technique. In 35 subjects, we also applied the forearm perfusion technique to quantify the responses of the forearm vascular bed to 3 increasing doses of 2 endothelium-dependent vasodilators (acetylcholine and bradykinin) and 1 endothelium-independent vasodilator (sodium nitroprusside). The D allele of the ACE gene was associated with a significant blunting (Δ=26%) of endothelium-dependent vasodilation in the femoral artery (P=0.02) but not in the brachial artery (P=0.55) or in the forearm microcirculation (P=0.70 to 0.80). Endothelium-independent vasodilation was unaffected by the ACE genotype. In young healthy humans, the D allele of the ACE gene is associated with selective endothelial dysfunction of the femoral artery. It remains to be determined whether this association discloses a causal role in vascular, particularly peripheral artery, disease. (Arterioscler Thromb Vasc Biol. 2001;21:1313-1319.)

Key Words: ACE/angiotensin receptors cardiovascular disease endothelium/vascular type/nitric oxide

Angiotensin-converting enzyme (ACE) catalyzes the conversion of angiotensin I to angiotensin II as well as the catabolism of bradykinin. An insertion (I)/deletion (D) polymorphism within the ACE gene exerts a codominant effect on plasma ACE levels in normal people. Because increased ACE activity can play a detrimental role in cardiovascular diseases and diabetic vascular complications, the ACE gene has been extensively scrutinized to demonstrate or refute its role in human disease, but with controversial findings.

In complex diseases, such as atherosclerosis, hypertension, and diabetes, the pathogenetic role of single genes may be difficult to gauge, unless one reverts to the study of simpler phenotypes, which (according to epidemiological and/or pathophysiological evidence) can be regarded as harbingers of disease. Endothelial function is one of such phenotypes because (1) endothelium is involved in several stages of atherogenesis, (2) all classic and most nonclassic risk factors of cardiovascular disease are associated with endothelial dysfunction, and (3) endothelial dysfunction precedes and predicts clinical macrovascular disease in human atherogenesis. Increased ACE activity may hamper endothelial function by increasing angiotensin II and, consequently, superoxide anion availability, thereby degrading NO. Alternatively, or concomitantly, an excess in ACE activity may accelerate bradykinin degradation and bradykinin-related NO effects.

In humans, Celermajer et al reported no relationship between ACE gene alleles and endothelium-dependent vasodilation in the brachial artery. Perticone et al found a blunting effect of the D allele on acetylcholine-induced vasodilation in the forearm microvasculature of hypertensive patients but not of healthy controls. Butler et al reported that the ACE gene D allele in homozygosity was associated not only with reduced acetylcholine-induced vasodilation but also with depressed nitroprusside-induced (ie, endothelium-independent) vasodilation in healthy people. Thus, their defect in vasomotion may not have been necessarily due to endothelial dysfunction. Finally, van Dijk et al found no differences in bradykinin-induced vasodilation in 8 II and 8 DD normotensive male individuals.

In our laboratory, in young individuals with no known risk factors for cardiovascular disease, endothelium-dependent vasodilations in the forearm microcirculation (acetylcholine-
induced), in the brachial artery, and in the femoral artery are not well correlated with each other (authors’ unpublished data), suggesting that in the absence of known cardiovascular risk factors, different determinants act on the vasodilatory responses triggered by these 3 endothelia. If the ACE gene is 1 of these determinants, it may exert differential effects on different endothelia.

Therefore, the present investigation was undertaken to study the association between ACE gene polymorphism and endothelium-dependent vasodilatation in the brachial and in the common femoral arteries of young healthy humans. In a smaller group of subjects, we also tested the effects of the ACE gene D/I alleles on forearm microvascular reactivity to 2 endothelium-dependent vasodilators (ie, acetylcholine and bradykinin) and 1 endothelium-independent vasodilator (sodium nitroprusside). Bradykinin was used because of the recent evidence showing a role of ACE gene alleles in mediating unrelated individuals were recruited for the present study. Each subject gave informed written consent before participating in the study, which was approved by the Human Investigation Committee of the Verona City Hospital.

After a preliminary screening, each subject attended 2 separate sessions. On the first occasion, baseline cardiovascular and humoral parameters were measured. On the second occasion, the noninvasive vascular tests were carried out. Thirty-one Italian volunteers came back on a third occasion to perform the noninvasive vascular tests. Thirty-five (4 of non-Italian descent) subjects participated in this protocol. Their characteristics are summarized in Table 1. Endothelium-dependent vasodilation (flow-mediated dilation induced by distal posts ischemic hyperemia) and endothelium-independent vasodilation (flow-mediated dilation induced by 400 µg sublingual nitroglycerin) were measured in the right common femoral artery, as previously described26,27 and in the brachial artery (84 subjects) during the same session.

Global quantitative indexes of flow-dependent and endothelium-independent vasodilation were obtained by calculating the area under the curve of change in vessel diameter as a function of time (from 0 to 8 minutes after the beginning of distal posts ischemic hyperemia and from 0 to 5 minutes after nitroglycerin administration), expressed as absolute values and also as percent changes over the baseline vessel diameter.26,27 Please refer online to http://atvb.ahajournals.org for the statistical power of the noninvasive and invasive vascular tests.28

### Methods

For the online unabridged version of this section, please refer to http://atvb.ahajournals.org.

### Subjects

Ninety-two young (aged between 20 and 29 years) normal nonsmoking unrelated individuals were recruited for the present study. Each subject gave informed written consent before participating in the study, which was approved by the Human Investigation Committee of the Verona City Hospital.

After a preliminary screening, each subject attended 2 separate sessions. On the first occasion, baseline cardiovascular and humoral parameters were measured. On the second occasion, the noninvasive (n=88) or the invasive (n=4) vascular tests were carried out. Thirty-one Italian volunteers came back on a third occasion to undergo the invasive vascular tests.

### Noninvasive Vascular Tests

Eighty-eight Italian subjects participated in this protocol. Their characteristics are summarized in Table 1. Endothelium-dependent vasodilation (flow-mediated dilation induced by distal posts ischemic hyperemia) and endothelium-independent vasodilation (flow-mediated dilation induced by 400 µg sublingual nitroglycerin) were measured in the right common femoral artery, as previously described26,27 and in the brachial artery (84 subjects) during the same session.

Global quantitative indexes of flow-dependent and endothelium-independent vasodilation were obtained by calculating the area under the curve of change in vessel diameter as a function of time (from 0 to 8 minutes after the beginning of distal posts ischemic hyperemia and from 0 to 5 minutes after nitroglycerin administration), expressed as absolute values and also as percent changes over the baseline vessel diameter.26,27 Please refer online to http://atvb.ahajournals.org for the statistical power of the noninvasive and invasive vascular tests.28

### Invasive Vascular Tests (Forearm Perfusion Study)

Thirty-five (4 of non-Italian descent) subjects participated in this protocol. Their characteristics are summarized in Table 2. The forearm perfusion studies were performed as previously described29,30 and lasted ~180 minutes. Catheters were introduced percutaneously into the brachial artery and retrogradely into an ipsilateral deep vein of the nondominant forearm. Acetylcholine, bradykinin, and sodium nitroprusside were infused at 3 incremental doses (acetylcholine 3, 9, and 30 µg · min⁻¹ · kg⁻¹ forearm tissue; bradykinin 40, 120, and 400 ng · min⁻¹ · kg⁻¹ forearm tissue; and sodium nitroprusside 1, 3, and 10 µg · min⁻¹ · kg⁻¹ forearm tissue). Forearm blood flow was measured by the intravascular indicator (Infracyanine Green Dye, SERB) dilution technique.29,30 Data were normalized per kilogram of forearm tissue.

### Analytical Methods

Please refer online to http://atvb.ahajournals.org and to Solini et al31 and Sambrook et al32 for analytical methods.

### Statistical Analysis

All data are presented as mean±SEM. All comparisons were performed by 1-way ANOVA (for repeated measures in the case of the forearm vascular responses), with or without adjustments for covariates as specified in the text and with repeated contrasts between adjacent groups (ie, DD versus ID, and ID versus II), to protect against multiple testing. All statistics were computed with the SPSS 9.0 software. Statistical significance was declared at P<0.05.

### Table 1. Demographic, Anthropometric, Cardiovascular, and Humoral Parameters of Noninvasive Vascular Study Subjects Stratified According to ACE Gene Polymorphism Status (II, ID, and DD)

<table>
<thead>
<tr>
<th></th>
<th>II</th>
<th>ID</th>
<th>DD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>18</td>
<td>39</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Sex (male/female), n</td>
<td>10/8</td>
<td>14/25</td>
<td>14/17</td>
<td>0.42</td>
</tr>
<tr>
<td>Age, y</td>
<td>23.9±0.46</td>
<td>23.9±0.35</td>
<td>23.5±0.38</td>
<td>0.76</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>21.9±0.53</td>
<td>22.0±0.44</td>
<td>22.6±0.50</td>
<td>0.54</td>
</tr>
<tr>
<td>W/H ratio</td>
<td>0.81±0.02</td>
<td>0.79±0.02</td>
<td>0.78±0.01</td>
<td>0.39</td>
</tr>
<tr>
<td>Systolic pressure, mm Hg</td>
<td>119±1.9</td>
<td>116±1.8</td>
<td>119±1.7</td>
<td>0.46</td>
</tr>
<tr>
<td>Diastolic pressure, mm Hg</td>
<td>69.8±1.2</td>
<td>67.5±0.98</td>
<td>68.1±1.0</td>
<td>0.39</td>
</tr>
<tr>
<td>Mean pressure, mm Hg</td>
<td>83.5±1.5</td>
<td>81.5±1.2</td>
<td>83.2±1.3</td>
<td>0.49</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>66±3</td>
<td>65±2</td>
<td>63±2</td>
<td>0.76</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>2.36±0.13</td>
<td>2.66±0.12</td>
<td>2.53±0.10</td>
<td>0.26</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.54±0.08</td>
<td>1.54±0.07</td>
<td>1.70±0.07</td>
<td>0.18</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.64±0.05</td>
<td>0.87±0.06</td>
<td>0.85±0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>25.5±1.8</td>
<td>35.0±3.2</td>
<td>31.5±2.8</td>
<td>0.13</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>4.68±0.08</td>
<td>4.85±0.06</td>
<td>4.72±0.06</td>
<td>0.16</td>
</tr>
<tr>
<td>Plasma ACE, U/L</td>
<td>21.7±1.2</td>
<td>30.8±1.6</td>
<td>39.2±2.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; W, waist; H, height; LDL-C, LDL cholesterol; and HDL-C, HDL cholesterol. Values are mean±SEM.
TABLE 2. Demographic, Anthropometric, Cardiovascular, and Humoral Parameters of Forearm Vascular Study Subjects Stratified According to ACE Gene Polymorphism Status (II, ID, and DD)

<table>
<thead>
<tr>
<th></th>
<th>II</th>
<th>ID</th>
<th>DD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>9</td>
<td>18</td>
<td>8</td>
<td>0.70</td>
</tr>
<tr>
<td>Sex (male/female), n</td>
<td>5/4</td>
<td>10/8</td>
<td>6/3</td>
<td>0.08</td>
</tr>
<tr>
<td>Age, y</td>
<td>24.0±0.6</td>
<td>24.9±0.6</td>
<td>22.5±0.8</td>
<td>0.01</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.0±0.9</td>
<td>23.0±0.6</td>
<td>24.3±0.6</td>
<td>0.45</td>
</tr>
<tr>
<td>W/H ratio</td>
<td>0.83±0.03</td>
<td>0.85±0.02</td>
<td>0.80±0.03</td>
<td>0.38</td>
</tr>
<tr>
<td>Systolic pressure, mm Hg</td>
<td>120±2.3</td>
<td>114±3.0</td>
<td>120±3.3</td>
<td>0.26</td>
</tr>
<tr>
<td>Diastolic pressure, mm Hg</td>
<td>68.9±2.1</td>
<td>64.9±1.3</td>
<td>68.8±2.2</td>
<td>0.15</td>
</tr>
<tr>
<td>Mean pressure, mm Hg</td>
<td>83.9±1.9</td>
<td>79.6±1.8</td>
<td>83.4±2.5</td>
<td>0.24</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>62±3</td>
<td>59±3</td>
<td>60±2</td>
<td>0.79</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>2.39±0.26</td>
<td>2.50±0.13</td>
<td>2.43±0.25</td>
<td>0.91</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.42±0.09</td>
<td>1.21±0.07</td>
<td>1.70±0.12</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.73±0.12</td>
<td>0.82±0.11</td>
<td>0.84±0.07</td>
<td>0.50</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>25.8±2.7</td>
<td>31.8±2.4</td>
<td>26.6±3.6</td>
<td>0.24</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>4.68±0.09</td>
<td>5.01±0.09</td>
<td>4.83±0.10</td>
<td>0.07</td>
</tr>
<tr>
<td>Plasma ACE, U/L</td>
<td>23.8±1.7</td>
<td>32.7±2.7</td>
<td>42.8±3.7</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

Results

Baseline Characteristics

Baseline data of the subjects participating in the noninvasive vascular tests are presented in Table 1. Baseline data of the subjects participating in the forearm study are presented in Table 2. In both sets of data, there were no differences in blood pressure, cholesterol, triglyceride, insulin, or glucose levels between the 3 ACE gene groups. However, in the group participating in the forearm vascular study, HDL cholesterol was higher in the DD group than in the ID group (P<0.01). The D/I allele frequencies were 0.574/0.426 and 0.486/0.514 in the noninvasive study and in the forearm study, respectively, resulting in the following genotype distribution: DD 35.6%, ID 43.7%, and II 20.7% in the group volunteering in the noninvasive vascular study and DD 22.9%, ID 51.4%, and II 25.7% in the group participating in the forearm study, respectively, moving from the II to the ID and to the DD group (P<0.001 by ANOVA).

Noninvasive Vascular Tests

Endothelium-independent vasodilation was positively correlated with endothelium-dependent vasodilation in the femoral artery (r=0.32, P<0.01) and in the brachial artery (r=0.23, P<0.05). Therefore, statistical analysis of endothelium-dependent vascular responses was carried out with endothelium-independent vasodilation as a covariate.

Similar postischemic accelerations in blood flow were achieved in the 3 groups in the brachial and in the common femoral artery (Table 3), thereby exposing the endothelium to a comparable stimulus. Endothelium-independent vasodilation was not statistically different in the 3 groups in either artery (Figures 1 and 2). The D allele, in homozygosity and in heterozygosity, was associated with a similar reduction in endothelium-dependent vasodilation in the common femoral artery (Figure 1, P<0.02 by ANOVA). Endothelium-dependent vasodilation was significantly lower in the DD than in the II subjects (P<0.01). Adjusting for basal arterial diameter, sex, body mass index, lipids, blood pressure,

TABLE 3. Femoral and Brachial Artery Baseline Diameter and Peak Flow Velocity During Endothelium-Dependent Vasodilation Test Stratified According to ACE Genotype Status

<table>
<thead>
<tr>
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<th>II</th>
<th>ID</th>
<th>DD</th>
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</thead>
<tbody>
<tr>
<td>Femoral artery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline diameter, mm</td>
<td>8.72±0.28</td>
<td>8.26±0.19</td>
<td>8.18±0.19</td>
<td>0.28</td>
</tr>
<tr>
<td>Peak flow velocity, cm/s</td>
<td>20.7±1.7</td>
<td>24.1±2.7</td>
<td>23.2±1.9</td>
<td>0.37</td>
</tr>
<tr>
<td>Brachial artery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline diameter, mm</td>
<td>4.01±0.15</td>
<td>3.77±0.11</td>
<td>3.78±0.10</td>
<td>0.37</td>
</tr>
<tr>
<td>Peak flow velocity, cm/s</td>
<td>15.1±1.6</td>
<td>14.3±1.4</td>
<td>16.3±1.7</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
glomerular filtration but not the extraglomerular microcirculation is associated with significant blunting of flow-mediated endothelium-dependent vasodilation in the common femoral artery of young healthy subjects (Figure 1). To the best of our knowledge, this is the first evidence of a selective loss of endothelial function associated with ACE gene polymorphism in healthy humans.

The D allele of the ACE gene exerts a detrimental effect on endothelium-dependent vasodilation in subjects with established cardiovascular disease in vitro and in vivo. In healthy subjects, the results have been less concordant. Flow-mediated vasodilation of the brachial artery was unaffected by the ACE gene polymorphism, whereas acetylcholine-induced and sodium nitroprusside–induced vasodilation of forearm microcirculation was found to be reduced in subjects with the DD alleles in a study performed in British individuals but was not found to be reduced in another report involving Italian individuals. When bradykinin was selected as endothelium-dependent vasodilator, no differences were found between II and DD subjects. Anyhow, the finding in the British study cannot be regarded as evidence of endothelial dysfunction; it is compatible with the idea that a state of NO resistance exists in the forearm microcirculation of people with the DD genotype. Indeed, in the present study, Italian individuals with comparable forearm sensitivity to the NO donor sodium nitroprusside, irrespective of the ACE gene status, displayed no effects of I/D polymorphism on acetylcholine- and bradykinin-induced vasodilation (Figure 3), thereby confirming and extending 2 previous studies.

The negative finding with bradykinin (the present study and that of Van Dijk et al) is of special note, because catabolism of bradykinin to bradykinin fragment 1-5 across the human forearm is significantly accelerated in carriers of the D allele. However, the increased generation of bradykinin fragment 1-5 was not accompanied by a decreased bradykinin concentration downstream from the forearm vascular bed, thereby implying that the concentration of exogenous bradykinin at its receptor may not be heavily influenced by the D allele in the forearm vascular bed.

Finally, in the same individuals displaying a femoral endothelial defect associated with the D allele, we confirm in Figure 2 that endothelium-dependent vasodilation of the brachial artery is not influenced by ACE gene polymorphism. Taken together, all data point out that endothelial function is governed by different factors according to vascular bed and to type of artery. Site-related differences in endothelium-dependent and -independent vasodilation of human vessels have already been reported in the past and perhaps might be related, in part, to variations in the ACE activity of the vascular wall, which, however, may not be reflected by the conversion rate of circulating angiotensin I to angiotensin II.

There are several potential mechanisms linking ACE genotype and arterial endothelium (dys)function. First, in-
creased ACE activity, as seen in association with the D allele, may reduce bradykinin activity, which in turn would depress receptor-mediated production of several endothelium-derived relaxing factors, including, but not limited to, NO. \textsuperscript{19} Second, angiotensin II stimulates the activity of the endothelial NADH/NADPH oxidase and superoxide anion production, \textsuperscript{18} which causes endothelial damage through several mechanisms. Additional possible effects of angiotensin II are stimulation of preproendothelin gene transcription, facilitation of endothelin release, \textsuperscript{37} and enhanced production of prostaglandin H (PGH\textsubscript{2}). \textsuperscript{38} The relevance of a putative intracrine renin-angiotensin system \textsuperscript{39} to the vascular endothelium is unknown.

Our findings suggest that ACE genotype and, presumably, activity are of primary relevance only to femoral artery endothelium in healthy humans (Figure 1). Some implications should be examined.

First of all, the femoral, but not brachial, artery is a target of clinically relevant atherosclerosis. Thus, the ACE gene D allele might be a genetic independent indicator of increased susceptibility to peripheral artery disease. In patients with renovascular hypertension, the D allele is a significant risk susceptibility to peripheral artery disease. In patients with angiotsin II antagonists.

Some limitations of the present study need be pointed out. First, our sample was not drawn from a population-based survey; thus, we cannot rule out the possibility of an inadvertent selection bias. Second, this is a pure association study; results may reflect unsuspected an underlying population admixture of Italian people and not a causal linkage between gene and function. Third, our findings cannot be considered as a proof that ACE gene polymorphism does not at all influence endothelial function of the brachial artery and of the forearm microcirculation because, as detailed in Methods (please refer online to http://atvb.ahajournals.org), the present study did not have the statistical power to disclose quantitatively small effects. Indeed, ACE inhibition can improve endothelial function even in healthy individuals, a finding consistent with the idea that ACE gene can influence brachial vasomotility. \textsuperscript{45} Fourth, the phenotype (i.e., endothelium-dependent vasodilation in the femoral artery) associated with the ACE genotype (Figure 1) showed no apparent gene dosage effect, a pattern consistent with either a lack of statistical power or with a dominant effect of the D allele in modulating femoral artery endothelium-dependent vasodilation. Fifth, a recent detailed study of sequence variation in the human ACE gene has shown that the I/D polymorphism is in absolute linkage disequilibrium with 17 variant sites of the gene, thereby generating 2 distinct clades. The deletion (D) clade shows a further major genetic subdivision, implying the need of a more detailed genetic analysis of the traits associated with the D allele. \textsuperscript{46}
Dose-response curve of forearm blood flow to graded intra-arterial doses of acetylcholine (top), bradykinin (middle), and sodium nitroprusside (bottom) in young healthy subjects with ACE II (n=9), ID (n=18), and DD (n=8) genotypes.

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References
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