The Insertion/Deletion Polymorphism of the Angiotensin-Converting Enzyme Gene Determines Coronary Vascular Tone and Nitric Oxide Activity

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OBJECTIVES
We investigated whether the insertion/deletion (I/D) polymorphism in the angiotensin-converting enzyme (ACE) gene modulates vasomotor tone and endothelial function.

BACKGROUND
The deletion allele of the ACE I/D polymorphism has been associated with increased incidence of cardiovascular pathology. The risk is synergistically increased in patients who also possess the C allele at position 1,166 of the angiotensin type I (AT1) receptor gene.

METHODS
In 177 patients with coronary atherosclerosis or its risk factors, we investigated endothelial function with intracoronary acetylcholine (ACH), endothelium-independent smooth muscle function with sodium nitroprusside (SNP) and basal nitric oxide activity with L-NG monomethyl arginine.

RESULTS
Compared with ACE II genotype, patients with the ACE DD genotype had lower coronary microvascular and epicardial responses with SNP (coronary blood flow increase 196 ± 26% vs. 121 ± 11%, p = 0.003, and diameter increase 21.9 ± 2% vs. 17 ± 1%, p = 0.03, ACE II vs. DD, respectively). L-NG monomethyl arginine induced greater constriction in patients with the ACE DD compared with ACE II genotype (coronary blood flow –10 ± 4% vs. 11 ± 5%, p = 0.003, ACE DD vs. II and diameter constriction –6.3 ± 1.2% vs. –1.9 ± 1.2%, p = 0.01, respectively, in patients with atherosclerosis). No difference in ACH-mediated vasoemotion was detected between the three ACE genotypes. The AT1 receptor polymorphism did not influence responses to either SNP or ACH.

CONCLUSIONS
Patients possessing the D allele of the ACE gene have increased vascular smooth muscle tone. The enhanced tone appears to be counterbalanced by an increase in basal nitric oxide activity in patients with atherosclerosis. (J Am Coll Cardiol 2000;36:1579–86) © 2000 by the American College of Cardiology

The insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme (ACE) gene has recently been identified as a possible risk factor for several cardiovascular disorders. The gene is located on chromosome 17, and the polymorphism is characterized by the presence (insertion [I]) or absence (deletion [D]) of a 287-base-pair alu repeat within intron 16. Because the polymorphism is located in an intron, it is believed to be a neutral marker in strong linkage disequilibrium with one or more unknown functional variants located in or close to the ACE gene (1,2). The three genotypes include ACE DD and II homozygotes and ID heterozygotes (3). Cambien and colleagues (4) were the first to report an increased frequency of the ACE D allele in patients with myocardial infarction (MI). They also observed a synergistic effect between the ACE D allele and the C allele of an adenine/cytosine (A/C) base substitution at position 1,166 of the angiotensin type I (AT1) receptor gene. The risk of MI appeared to be greatest in individuals with the ACE DD and AT1 receptor CC genotypes (5). Synergism between the ACE gene polymorphism and conventional risk factors such as smoking and hyperlipidemia has also been observed (6,7). Though some studies have noted the absence of an association (8–10), a recent meta-analysis confirmed that the presence of the ACE D allele was a risk factor for MI (11). Despite considerable interest in this genetic variant, few studies have explored the potential vascular mechanisms by which presence of the ACE D allele leads to these deleterious effects.

Abnormal epicardial coronary vasoconstriction and depressed microvascular dilation are early features of atherosclerosis (12,13). This results from endothelial cell dysfunction, which modulates smooth muscle function and contributes to the pathogenesis of MI and ischemia. Angiotensin-converting enzyme, a key component of the circulating and vascular renin-angiotensin systems promotes the synthesis of angiotensin II, an important regulator of vascular function (14). By stimulating the AT1 receptor, angiotensin II promotes vasoconstriction, both directly and through endothelin release or augmentation of sympathetic tone. Moreover, increased free radical generation by angiotensin II may also contribute to endothelial dysfunction (15–18). In addition, ACE also metabolizes locally synthesized bradykinin, which would promote vasoconstriction by reducing bradykinin-dependent nitric oxide (NO) release. This vasoconstriction is partly inhibited by angiotensin II–mediated release of NO from the endothelium (19–22).
Because individuals with the ACE D allele have higher plasma and tissue ACE levels, we hypothesized that the D allele of the ACE gene, by increasing local angiotensin II generation and decreasing bradykinin activity, may modulate coronary vascular tone (23–27).

Thus, the aims of our study were to investigate whether the presence of the ACE D allele was: a) associated with increased smooth muscle constrictor tone tested with sodium nitroprusside (SNP), b) a determinant of basal NO activity assessed by the response to L-N\textsuperscript{G} monomethyl arginine (L-NMMA) and c) a risk factor for endothelial dysfunction, as tested with acetylcholine (ACH), in both coronary conductance and resistance vessels. In view of the previous report that the ACE polymorphism was not a determinant of vasomotor function in normal subjects, we conducted this study in patients with one or more risk factors for atherosclerosis in whom there is evidence of increased tissue ACE activity (6,7,28). We also evaluated whether any association between the ACE gene polymorphism and vascular phenotype was modulated by the AT\textsubscript{1} receptor A/C gene polymorphism.

**METHODS**

**Study population.** We studied patients undergoing diagnostic cardiac catheterization for investigation of chest pain or abnormal noninvasive tests. Risk factors were defined as the presence of hypertension (blood pressure > 140/90), hypercholesterolemia (low-density lipoprotein cholesterol > 160 mg/dL), diabetes, current smoking or smoking in the previous year and age > 65 years. Patients with coronary artery disease (CAD) who either had atherosclerotic plaques or more severe stenoses in one or more of their coronary arteries were included in the group with atherosclerosis. Patients with recent MI, valvular heart disease or those treated with ACE inhibitors in the previous two weeks were excluded. All cardiac medications were withdrawn at least 48 h before the study, and aspirin or other cyclooxygenase inhibitors were discontinued seven days before. The study was approved by the National Heart, Lung, and Blood Institute Investigational Review Board, and informed consent was obtained from all patients.

**Protocol.** After diagnostic coronary angiography was performed, a 6F guide catheter was introduced into the proximal segment of a coronary artery, and blood flow velocity was measured using a 0.018 inch wire equipped with a Doppler crystal at its tip (Cardiometrics Flowwire, Cardiometrics, Endosonics Corp., Rancho Cordova, California). The Doppler flow wire was advanced into either the left main or the proximal segment of a major epicardial artery. The wire tip was carefully positioned in a segment of the vessel that was straight and free of any major branches 1 cm from the tip, that produced a characteristic and stable flow velocity signal, and that could be imaged without overlap from other vessels, thus allowing for quantitative measurements of the coronary artery diameter. Flow measurements were made in a coronary artery with <30% stenosis to ensure that microvascular function could be measured. All drugs were infused directly into the left main or the right coronary artery via the guide catheter at rates ranging between 1 and 2 ml/min. The dose of drugs administered was halved in the right coronary artery.

**Study 1.** In 177 patients (92 with coronary atherosclerosis and 85 with angiographically normal coronary arteries and risk factors for atherosclerosis) we evaluated the relationship between both the ACE and AT\textsubscript{1} receptor gene polymorphisms and the coronary vasomotor responses to ACH and SNP. After a 5-min infusion of dextrose 5% at 1 ml/min, measurement of coronary blood flow velocity and coronary angiography were performed and repeated after each intervention (13). Endothelium-dependent vasodilation was estimated by measuring coronary flow and epicardial responses to an infusion of intracoronary ACH at an estimated intracoronary concentration of 10\textsuperscript{-6} (mol/L). Ten minutes after performing the ACH measurements and repeat baseline measurements, endothelium-independent coronary smooth muscle function was estimated with intracoronary SNP given at 40 µg/min for 3 min.

**Study 2.** In a subgroup of 76 patients (38 with coronary atherosclerosis and 38 with normal coronary arteries with risk factors for atherosclerosis) we evaluated the relationship between the ACE I/D polymorphism and basal NO production. Baseline measurements were made after a 10-min infusion of dextrose 5%. This was followed by a 10-min infusion of L-NMMA (Clinalfa AG, Switzerland), a specific inhibitor of NO synthesis from L-arginine, at 64 µmol/min (1 ml/min).

**Measurement of coronary blood flow and diameter.** Coronary blood flow was derived from the coronary blood flow velocity and diameter measurements using the formula (π× average peak velocity×0.125×diameter\textsuperscript{2}) (13). Coronary vascular resistance was calculated as mean arterial pressure divided by coronary blood flow. For calculating flow, coronary artery diameter was measured in a 0.5 cm segment of vessel beginning 0.25 cm beyond the tip of the flow wire. Coronary angiograms were recorded using a cineangiographic system (Toshiba, Inc., Tustin, California), and quantitative angiography was performed with the
**Table 1.** Patient Characteristics for Each Genotype

<table>
<thead>
<tr>
<th>Study 1</th>
<th>DD</th>
<th>ID</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>59</td>
<td>84</td>
<td>34</td>
</tr>
<tr>
<td>ACE level (U/L)</td>
<td>13.4 ± 0.6</td>
<td>10.2 ± 0.7</td>
<td>7.0 ± 0.7</td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>55 ± 1.3</td>
<td>55 ± 1.3</td>
<td>58 ± 1.7</td>
</tr>
<tr>
<td>Positive Hx of smoking (%)</td>
<td>38 (64)</td>
<td>50 (60)</td>
<td>17 (50)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>213 ± 6</td>
<td>225 ± 5</td>
<td>224 ± 8</td>
</tr>
<tr>
<td>Low-density lipoprotein (mg/dL)</td>
<td>134 ± 6</td>
<td>147 ± 5</td>
<td>147 ± 7</td>
</tr>
<tr>
<td>High-density lipoprotein (mg/dL)</td>
<td>42 ± 2</td>
<td>44 ± 2</td>
<td>42 ± 2</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>190 ± 15</td>
<td>182 ± 19</td>
<td>185 ± 16</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>34 (58)</td>
<td>47 (56)</td>
<td>13 (38)</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>10 (17)</td>
<td>18 (21)</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Coronary atherosclerosis (%)</td>
<td>31 (53)</td>
<td>42 (50)</td>
<td>19 (56)</td>
</tr>
</tbody>
</table>

*p = 0.001, DD vs. II.
ACE = angiotensin-converting enzyme; Hx = history; SD = standard deviation.

**RESULTS**

**Study 1.** The frequencies of the ACE D and I alleles were 57% and 43%, respectively, and the AT1 receptor A and C alleles were 77% and 23%, respectively. These frequencies were similar to those reported in previous studies (4,5,28), and the genotype frequency did not deviate significantly from that predicted by the Hardy–Weinberg equilibrium. There were 102 (58%) men; mean age was 55 ± 1 years; 67 (38%) were hypertensive; 106 (60%) were current or previous smokers; 29 (16%) had diabetes; and the mean cholesterol level was 221 ± 3 mg/dL. As reported previously, ACE levels were significantly higher in patients with the D allele (23). The distribution of conventional risk factors for atherosclerosis and endothelial dysfunction was similar between the three groups of patients (Table 1).

**Genotype and coronary vascular response to SNP.** Baseline systemic blood pressure, coronary epicardial diameter and vascular resistance were similar in patients with all three ACE and AT1 receptor genotypes (Table 2). Sodium nitroprusside infusion reduced mean arterial pressure from...
110 ± 1 to 103 ± 1 mm Hg (p < 0.001), but the change was not significantly different between the genotype groups. There was a progressive decrease in coronary epicardial and microvascular responses to SNP in the presence of the ACE D allele, with the lowest response in patients homozygous for the D allele, intermediate response in those with the ACE ID genotype and greatest response in those homozygous for the I allele (p = 0.003 for flow and p = 0.085 for diameter by ANOVA, Fig. 1). Thus, compared with patients with the ACE DD genotype, those with ACE II genotype had greater increase in flow (196 ± 26% vs. 121 ± 11%, p = 0.003) and diameter (21.9 ± 2% vs. 17 ± 1%, p = 0.03) with SNP. In multivariate analysis, the presence of the ACE D allele (p = 0.007) and diabetes mellitus (p = 0.04) were independent predictors of the magnitude of increase in coronary blood flow with SNP.

The responses to SNP in patients with AT1 AA, AC and CC genotypes were similar (Fig. 2, Table 3). The analysis for the interaction between the two polymorphisms was performed in patients with the D allele of the ACE gene. There was no difference in the responses in individuals with the ACE D allele (DD or ID) who were homozygous for the A allele when compared to those possessing the C allele (AC and CC genotypes) of the AT1 receptor gene (Table 3).

**Figure 1.** Effects of acetylcholine and sodium nitroprusside on coronary vascular resistance, coronary blood flow and epicardial diameter according to angiotensin-converting enzyme genotype.

**Table 2.** Baseline Coronary and Systemic Hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Study 1</th>
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</thead>
<tbody>
<tr>
<td>DD</td>
<td>111 ± 2</td>
<td>112 ± 2</td>
<td>109 ± 3</td>
</tr>
<tr>
<td>ID</td>
<td>2.0 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>II</td>
<td>46 ± 4</td>
<td>47 ± 5</td>
<td>47 ± 9</td>
</tr>
<tr>
<td>Coronary blood flow (ml/min)</td>
<td>3.6 ± 0.4</td>
<td>3.7 ± 0.3</td>
<td>3.9 ± 0.5</td>
</tr>
<tr>
<td>Mean blood pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epicardial diameter (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary vascular resistance (mm Hg · ml⁻¹ · min⁻¹)</td>
<td></td>
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<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Study 2</th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DD</td>
<td>109 ± 2</td>
<td>112 ± 3</td>
<td>106 ± 3</td>
</tr>
<tr>
<td>ID</td>
<td>1.9 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>II</td>
<td>45 ± 8</td>
<td>60 ± 10</td>
<td>46 ± 6</td>
</tr>
<tr>
<td>Coronary blood flow (ml/min)</td>
<td>3.9 ± 0.7</td>
<td>3.2 ± 0.4</td>
<td>3.4 ± 0.5</td>
</tr>
<tr>
<td>Mean blood pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epicardial diameter (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary vascular resistance (mm Hg · ml⁻¹ · min⁻¹)</td>
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</table>

**Figure 2.** Effects of acetylcholine and sodium nitroprusside on coronary vascular resistance, coronary blood flow and epicardial diameter according to angiotensin type I receptor genotype.
to SNP. The ratio of the coronary blood flow increase with ACH compared with the increase with SNP was 1.16 ± 0.1, 1.06 ± 0.1 and 0.96 ± 0.1 in the ACE DD, ID and II genotypes, respectively (p = NS, by ANOVA). Similarly, the ratio of ACH versus SNP responses in the epicardial coronary arteries were not significantly different.

The responses to ACH were also not different in patients with the AT1 AA, AC and CC genotypes (Table 3, Fig. 2). An analysis for the interaction between the two polymorphisms, as described for SNP, was also performed for ACH responses, and no difference was found (Table 3).

Study 2: genotype and coronary vascular response to L-NMMA. The frequencies of the D and I alleles were 52% and 48%, and the frequencies of the ACE DD, ID and II genotypes were 29%, 46% and 25%, respectively, in this subgroup. Genotype frequency did not deviate significantly from that predicted by the Hardy–Weinberg equilibrium. Baseline systemic and coronary hemodynamics and the distribution of conventional risk factors for endothelial dysfunction were similar in patients with all three genotypes (Table 2).

L-NG monomethyl arginine infusion increased mean arterial pressure from 110 ± 2 to 117 ± 2 mm Hg (p < 0.001) in the total study population, and the magnitude of increase was similar in the three genotype subsets. The increase in coronary vascular resistance and the reduction in blood flow with L-NMMA was greater in patients with the ACE D allele (p = 0.07, by ANOVA for both, Fig. 3). Thus, the −7 ± 3% reduction in coronary blood flow in the ACE DD patients was higher than the ACE II patients in whom blood flow changed by 5 ± 4%, p = 0.003. Patients with the ACE ID genotype had an intermediate response. In multivariate analysis, the presence of the ACE D allele (p = 0.08) appeared to be a predictor of the magnitude of change in coronary blood flow with L-NMMA.

Although the trend toward a decreased epicardial response to L-NMMA in patients with the ACE II genotype did not reach statistical significance compared with those with the ACE DD genotype in the whole group (Fig. 3), this difference was significant in the subgroup of 37 patients with angiographic atherosclerosis in whom epicardial diameter decreased by 6.3 ± 1.2%, 5.0 ± 1.4% and 1.9 ± 1.2% in ACE DD, ID and II genotypes, respectively (p = 0.04, by ANOVA).

Table 3. Coronary Epicardial and Microvascular Responses According to the AT1 Receptor Gene Polymorphism

<table>
<thead>
<tr>
<th>Variables % Change</th>
<th>AA</th>
<th>AC</th>
<th>CC</th>
<th>ANOVA</th>
<th>D + AA</th>
<th>D + AC/CC</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium nitroprusside</td>
<td>Resistance</td>
<td>−53 ± 2</td>
<td>−58 ± 3</td>
<td>−51 ± 7</td>
<td>0.1</td>
<td>−51 ± 3</td>
<td>−53 ± 3</td>
</tr>
<tr>
<td></td>
<td>Flow</td>
<td>130 ± 8</td>
<td>164 ± 20</td>
<td>139 ± 36</td>
<td>0.2</td>
<td>125 ± 9</td>
<td>129 ± 14</td>
</tr>
<tr>
<td></td>
<td>Diameter</td>
<td>18.7 ± 1.1</td>
<td>18.1 ± 1.3</td>
<td>17.7 ± 2.5</td>
<td>0.9</td>
<td>17.8 ± 1.2</td>
<td>17.1 ± 1.3</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>Resistance</td>
<td>−41 ± 3</td>
<td>−49 ± 3</td>
<td>−41 ± 6</td>
<td>0.2</td>
<td>−41 ± 3</td>
<td>−48 ± 3</td>
</tr>
<tr>
<td></td>
<td>Flow</td>
<td>107 ± 10</td>
<td>146 ± 19</td>
<td>104 ± 31</td>
<td>0.1</td>
<td>105 ± 11</td>
<td>128 ± 15</td>
</tr>
<tr>
<td></td>
<td>Diameter</td>
<td>−1.0 ± 0.8</td>
<td>0.7 ± 1.2</td>
<td>−3.4 ± 2.4</td>
<td>0.2</td>
<td>−1.8 ± 0.9</td>
<td>1.0 ± 1.1</td>
</tr>
</tbody>
</table>

p value = D + AA vs. D + AC/AC.
ANOVA = analysis of variance, AA vs. AC vs. CC; AT1 = angiotensin type I.

**DISCUSSION**

To obtain insights into mechanisms underlying the association between the D allele and increased prevalence of vascular disease, we examined the effects of the ACE gene polymorphism on coronary endothelial and smooth muscle function. Endothelial function was studied using both L-NMMA, to estimate basal NO activity, and ACH, to estimate endothelial function upon pharmacologic stimulation. Smooth muscle function was assessed using an endothelium-independent dilator, SNP. Our study demonstrates that the presence of the ACE D allele: a) is

![Figure 3. Effects of L-NG monomethyl arginine (L-NMMA) on coronary vascular resistance, coronary blood flow and epicardial diameter according to angiotensin-converting enzyme genotype.](image_url)
associated with depressed vasodilation with SNP denoting increased vascular smooth muscle tone; b) is associated with enhanced vasoconstriction with L-NMMA confirming increased basal NO activity; and c) does not influence ACH responses, indicating that the ACE genotype may not be a determinant of stimulated endothelial function in the coronary circulation of patients with atherosclerosis or one of its risk factors. Furthermore, the 1,166 A/C AT1 receptor gene polymorphism does not influence coronary vascular endothelial or smooth muscle function.

**ACE deletion allele and basal constrictor tone.** A significant reduction in the dilator response of microvessels and epicardial arteries with SNP, a NO donor that directly stimulates smooth muscle production of cyclic guanylate monophosphate, was observed in patients with the D allele, compared with those with the ACE II genotype. This reduced vasodilation with SNP may reflect enhanced smooth muscle constrictor tone due to higher angiotensin II and lower bradykinin activity (25,27,29,30) in blood vessels of patients with the ACE D allele who have increased circulating and tissue ACE levels (23,24). In addition, these patients may also have increased smooth muscle constrictor tone due to angiotensin II–mediated endothelin release and stimulation of sympathetic activity (15,16). Since this increased smooth muscle constrictor tone in patients with the D allele was not associated with increased resting coronary vascular resistance and smaller epicardial diameters, it is likely that counter–regulatory endogenous release of dilating substances, possibly endothelium-derived, is also upregulated in these patients. This possibility is supported by our observation that basal NO activity was higher in individuals with the D allele.

**D allele and basal NO activity.** Presence of the ACE D allele was associated with greater microvascular and epicardial constriction with L-NMMA compared with those with the ACE II genotype. This may be a consequence of higher basal NO activity and/or increased vasoconstrictor tone in the coronary vasculature of individuals with the ACE D allele. The former mechanism is supported by a previous in vitro study in which higher basal NO activity was present in internal mammary artery segments from patients with CAD who possessed the ACE D allele (26). Furthermore, this is analogous to the recent finding that a high lipoprotein(a) level, another risk factor for cardiovascular disease, is associated with increased basal NO release (31). It is important to recognize that the variable stimulation of basal NO activity occurred despite the presence of multiple risk factors that are themselves associated with reduced NO activity (15). Multivariate analysis of determinants of basal NO activity (L-NMMA response) demonstrated that the ACE D allele tended to be an independent determinant of the L-NMMA response in the coronary microvasculature. This emphasizes the important additional role of the D genotype and, hence, tissue ACE activity on NO bioavailability. The mechanisms underlying this observation may be explained by the fact that angiotensin II itself stimulates NO release. Angiotensin II promotes NO release in a dose-dependent manner by activating NO synthase either via the AT1 or AT2 receptor stimulation (19,20,22). This raises the possibility that activation of the tissue renin-angiotensin system in atherosclerosis (32) may also regulate basal NO activity in humans so that greater angiotensin II activity in individuals with the ACE D allele would be expected to lead to higher basal NO release.

Alternatively, it is also likely that the greater response to L-NMMA may be due to the increased smooth muscle tone in patients with the D allele, as suggested by the diminished vasodilator response to SNP as discussed above. The lack of difference in basal coronary vascular tone in patients with different ACE I/D genotypes leads us to speculate that the increased smooth muscle tone is offset by increased production of endogenous vasodilators such as NO.

**ACE D allele and stimulated endothelial function.** Endothelial dysfunction is characterized by depressed microvascular and epicardial dilation in response to pharmacologic probes including ACH (13). In our study ACH-mediated microvascular and epicardial vasomotion was similar in all three genotype groups, indicating that the ACE gene polymorphism does not modulate endothelium–dependent vasomotor dysfunction in patients with one or more risk factors for atherosclerosis. Because smooth muscle tone as assessed by SNP, an NO donor, was increased in patients with the ACE D allele, it is important to interpret the unchanged response with ACH, that releases NO via the endothelium, in this population with caution. We therefore analyzed the response to ACH as a ratio of the response to SNP and found no statistical difference between the groups, confirming that stimulated endothelium–dependent vasomotion was not different between the groups. An explanation as to why the response to ACH is unaltered when the response to an endothelium–independent NO donor, SNP, is reduced in patients with the D allele may be that these patients had enhanced stimulated NO release. This possibility is consistent with the observed increase in basal NO activity. Alternatively, there may be increased release non–NO endothelium–derived relaxing factors in response to ACH, a release that compensates for the increased smooth muscle tone in patients with the ACE D allele.

Our findings are consistent with a previous study in individuals without risk factors for atherosclerosis in whom the ACE gene polymorphism also did not determine endothelial function, which was assessed by brachial artery dilation during reactive hyperemia (28). However, a recent study reported that individuals with the ACE DD genotype had higher plasma PAI-1 levels, von Willebrand factor and thrombomodulin, all markers of endothelial dysfunction (33,34). This raises the possibility that the ACE gene polymorphism may impair functions of the endothelium that are independent of factors that control vasomotion.

**AT1 receptor polymorphism and vasomotor function.** In our study the A/C polymorphism of the angiotensin AT1 receptor gene did not influence endothelial or smooth muscle function either directly or in synergy with the ACE.
gene. Because of the low frequency of the C allele, we were unable to determine the influence of this polymorphism on basal NO activity. Though the polymorphism has been previously associated with enhanced coronary vasoconstriction with methylergonovine (35), our data suggest that the receptor polymorphism is not a major determinant of vasomotor function in patients with atherosclerosis or its risk factors. This conclusion is consistent with the fact that the genetic variant is: a) located in the noncoding region and thus does not alter the amino acid sequence of the receptor protein, b) not a direct risk factor for coronary artery disease and c) only a weak risk factor for hypertension (5,36).

**Study limitations.** Although we did not evaluate the vasomotor responses to bradykinin here, in a subsequent study we observed that epicardial dilation with bradykinin is diminished in individuals with the ACE D allele compared with those with the II genotype (37). Together with the present study, these data indicate that the ACE gene polymorphism modulates kinin but not muscarinic receptor mediated endothelial function. We did not investigate the interaction between the presence of the ACE D allele and individual risk factors for CAD in determining vascular function, because of the limited and selected population. We also did not investigate whether the ACE I/D polymorphism affected other functions of the endothelium, such as adhesion molecule expression, lipid oxidation and PAI-1 synthesis, which are all sensitive to angiotensin II. It is possible that the deleterious effects of the D allele in the ACE gene and the C allele of the AT1 receptor gene may be mediated through one or more of these mechanisms.

**Conclusions and implications.** For the first time, to our knowledge, we demonstrated the influence of a patient’s genotype on coronary vasomotor function, which reveals the complex inter-relationships between vasoconstrictors and vasodilators and activation of compensatory pathways. We show that in patients with either atherosclerosis or its risk factors, the D allele of the ACE gene is associated with greater basal NO activity, possibly in compensation for the increased vascular smooth muscle tone, which may be secondary to increased local angiotensin II and reduced bradykinin activities. Thus, the ACE I/D polymorphism may predispose to hypertension, MI and coronary artery spasm (37) (ref. 37 published in this issue of the Journal), not by inducing endothelial injury but by promoting smooth muscle vasoconstriction. This conclusion is supported by the recent observation that individuals with the ACE DD genotype have greater systemic constrictor response to phenylephrine and increased angiotensin II–induced potentiation of phenylephrine-mediated constriction of arterial segments in vitro compared with those with the ACE ID/II genotypes (39). Finally, the AT1 receptor A/C polymorphism is not a determinant of coronary vasomotor function and does not modulate the effects of the ACE genotype on the vascular phenotype.

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**REFERENCES**


