ACE D/I Polymorphism and Incidence of Post-PTCA Restenosis
A Prospective, Angiography-Based Evaluation

Robert Y.L. Zee, Antonio Fernandez-Ortiz, Carlos Macaya, Emilio Pintor, Klaus Lindpaintner, Arturo Fernandez-Cruz

Abstract—Early restenosis is the major complication of percutaneous transluminal coronary angioplasty (PTCA), occurring in ≈30% of all initially successful procedures. The D/I polymorphism of the ACE gene, which has variably been reported to represent a risk factor for manifestations of ischemic heart disease, has recently been implicated in the pathophysiology of restenosis after PTCA by some investigators but not by others. All studies conducted thus far involved relatively small sample sizes. We investigated the possible association of ACE D/I genotype and post-PTCA restenosis in a large, prospective sample of patients followed by quantitative coronary angiography. The ACE D/I gene polymorphism was characterized in a cohort of 779 patients, of whom 342 (cases) had developed restenosis (as defined by >50% loss of lumen compared with immediate postprocedural results) at repeat quantitative coronary angiography at 6 months after PTCA. Allele frequencies for the ACE D and I alleles were 0.58 and 0.42 in cases and 0.58 and 0.42 in control subjects. All observed genotype frequencies were in Hardy-Weinberg equilibrium. There was no evidence for an association between genotype and restenosis or degree of lumen loss. The data from this largest study of its kind conducted so far provide no evidence for an association of the ACE D/I allelic polymorphism with incidence of restenosis after PTCA. On the basis of the power of this study, we conclude that in a general population, the ACE D/I polymorphism is not a useful marker to assess risk of post-PTCA restenosis. (Hypertension. 2001;37:851-855.)

Key Words: angiotensin-converting enzyme □ angioplasty □ polymorphism □ genetics □ risk factors

The utility of percutaneous transluminal coronary angioplasty (PTCA) is limited by a high incidence of restenosis.1 Experimental and clinical studies have implicated vessel remodeling in restenosis after PTCA.2 Although a number of factors potentially promoting remodeling are being discussed, the exact molecular and cellular mechanisms of post-PTCA remodeling in humans remain, however, largely unknown. None of a large number of known risk factors for atherosclerosis or ischemic cardiovascular disease except for diabetes mellitus3 has been found to be associated with the occurrence of this complication. Genetic factors are believed to be an important reason for interindividual differences in treatment response. Thus, studies correlating such factors with the incidence of this complication might shed new light on its pathogenesis; such information, in turn, would provide us with tools for prediction and possibly suggest novel therapeutic/preventive approaches.

ACE, with its demonstrated growth-stimulating effects on vascular smooth muscle cells, represents a possible candidate that may contribute to restenosis. An insertion/deletion polymorphism in the gene encoding ACE has consistently been found associated with differential plasma ACE levels and has attracted widespread interest in recent years, based on conflicting reports of association with various cardiovascular disease states.4 Among them, post-PTCA restenosis has been linked to this marker in some5-10 but not other studies.11-13 All of these studies were of moderate size only, with numbers of cases ranging as low as 17,6 most without quantitative coronary angiography (QCA) assessment,5,7,9,10,13 and commonly based on retrospectively assembled cohorts.5,6,9,11,12 Given the limited power of each of these studies and their individual shortcomings, a more conclusive assessment and a resolution of the contradictory answers they provided has so far not been possible.

We recently had the opportunity to conduct a study in post-PTCA patients that not only avoided the limitations mentioned by being prospective and based on QCA measurements at baseline and 6 months after PTCA but that also used a sample >3 times as large as in the largest study heretofore reported.12

Received February 16, 2000; first decision March 20, 2000; revision accepted August 30, 2000.
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Methods

Study Design

Between March 1995 and March 1997, we obtained permission of consecutive patients who had undergone successful PTCA at the Hospital San Carlos, Universidad Complutense in Madrid, Spain, to perform an angiographic follow-up examination 6 months later, by having an appropriate, comprehensive informed consent form signed. QCA was performed before PTCA, immediately after PTCA (demonstrating the effect of the procedure), and at follow-up cardiac catheterization 6 months later. Ultimately, 779 patients with complete clinical and angiographic follow-up were enrolled, for whom blood samples were available for the present investigation. With a >50% reduction in the luminal diameter at the site of previous PTCA as compared with the immediate postangioplasty angiographic record as the criterion to define restenosis, there were 342 cases (with a total of 378 restenotic lesions).

QCA analysis was carried out off-line independently by 2 experienced operators who were blinded to patients’ clinical or procedural characteristics, using a commercially available, previously validated, interactive automatic edge-detection computer program (ARTREK, Quantim 2001, QCS Inc. ImageComm System Inc.). Disagreements (<1%) were resolved by a further joint reading. Two orthogonal, nonforeshortened angiographic views that optimized separation of the lesion and its proximal coronary segment were selected for analysis. The angioplasty-treated coronary segment was always documented before PTCA, immediately after PTCA, and at 6-month follow-up, with the same views. All analyses were performed on images taken after the administration of intracoronary nitroglycerin.

ACE D/I Genotype Determination

A detailed description of the protocol used to determine ACE D/I genotype has previously been published. In brief, the D and I alleles were identified on the basis of polymerase chain reaction (PCR) amplification of the respective fragments from exon 16 of ACE and by subsequent electrophoretic size fractionation and visualization after staining with ethidium bromide. Because the D allele tends to be preferentially amplified in heterozygotes, all samples determined to be DD were subjected to a second independent PCR amplification with a primer pair that recognizes an insertion-specific sequence to ensure accurate genotyping. To confirm genotype assignment, the PCR procedure was performed on all samples on 2 separate occasions. PCR results were scored blinded as to case-control status.

Statistical Analysis

Alleles and genotype frequencies among cases and control subjects were counted and compared with Hardy-Weinberg predictions by $\chi^2$ analysis. Crude quantitative data on fractional restenosis were evaluated by analysis for trend (additive model, DD versus DI versus II) or $\chi^2$ analysis (dominant and recessive models, respectively). In addition, multivariate logistic (with dichotomous dependent variables) and linear (parameters: acute gain, ie, the difference between obstruction diameter after and before angioplasty; late loss, ie, the ratio of late loss to acute gain) regression analyses were performed, adjusting for age, gender, body mass index (BMI), blood pressure, total serum cholesterol, smoking habit, diagnosis of diabetes mellitus, angiographic and occlusion characteristics, and family history of myocardial infarction (MI). Odds ratios were calculated as a measure of the association of the ACE genotype with the phenotype of restenosis, with the effects of the D allele assumed to be additive (with scores of 0, 1, and 2 assigned for II, DI, and DD, respectively), dominant (with scores of 0 for II and 1 for DI and DD combined), or recessive (with scores of 0 for II and DI combined, and 1 for DD). For each odds ratio, we calculated 95% confidence intervals. A 2-tailed probability value of 0.05 was considered to represent a statistically significant result.

### Table 1. Baseline Characteristics of the Study Population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases (n=342)</th>
<th>Control Subjects (n=437)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y ± SD</td>
<td>58.9±9.6</td>
<td>58.2±10.2</td>
<td>0.34</td>
</tr>
<tr>
<td>Men/women</td>
<td>305/37</td>
<td>378/59</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m² ± SD</td>
<td>26.7±3.5</td>
<td>26.9±3.4</td>
<td>0.53</td>
</tr>
<tr>
<td>Hypertension &gt;160/90 mm Hg</td>
<td>149 (46.5%)</td>
<td>183 (41.8%)</td>
<td>0.22</td>
</tr>
<tr>
<td>Hypercholesterolemia &gt;220 mg/dL</td>
<td>146 (42.8%)</td>
<td>201 (46.0%)</td>
<td>0.38</td>
</tr>
<tr>
<td>Smokers</td>
<td>82 (24%)</td>
<td>106 (24.3%)</td>
<td>0.93</td>
</tr>
<tr>
<td>Diabetes</td>
<td>68 (19.9%)</td>
<td>57 (13%)</td>
<td>0.011</td>
</tr>
<tr>
<td>Family history of MI, age &lt;60 y</td>
<td>78 (22.8%)</td>
<td>87 (19.9%)</td>
<td>0.33</td>
</tr>
<tr>
<td>Previous MI</td>
<td>156 (45.6%)</td>
<td>189 (43.2%)</td>
<td>0.51</td>
</tr>
<tr>
<td>Stable angina</td>
<td>64 (18.7%)</td>
<td>85 (19.5%)</td>
<td>0.79</td>
</tr>
<tr>
<td>Unstable angina</td>
<td>221 (66.4%)</td>
<td>267 (61.1%)</td>
<td>0.31</td>
</tr>
</tbody>
</table>

AHA/ACC: American Heart Association/American College of Cardiology classifications; total occlusion, TIMI (Thrombolysis In Myocardial Infarction Trial) flow grade 0 or 1.

### Results

#### Characteristics of the Study Population

Baseline characteristics of the study population are shown in Table 1. There were no significant differences among the cases (restenosis) and the control subjects (no restenosis) with regard to age, blood pressure status, plasma lipid profile, smoking status, or family history of coronary heart disease. As expected, cases were more likely to have diabetes ($\chi^2_{2df}=6.66, P=0.011$).

#### Alleles and Genotype Frequencies

Allele frequencies for D and I alleles were 0.58 and 0.42 in case subjects and 0.58 and 0.42 in control subjects, respectively. The observed genotype frequencies (Table 2) did not deviate significantly from the Hardy-Weinberg equilibrium in control subjects ($\chi^2_{1df}=0.28, P=0.65$), cases ($\chi^2_{1df}=0.059, P=0.85$) or the whole study group ($\chi^2_{1df}=0.14, P=0.80$). No overall difference in genotype distribution was seen among cases and control subjects ($\chi^2_{2df}=0.23, P=0.89$).

#### Genotype-Restenosis Association

Analysis of the crude unadjusted data by categorical assignment of case status to subjects showing >50% lumens loss in

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases (n=342)</th>
<th>Control Subjects (n=437)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD</td>
<td>113 (33.0%)</td>
<td>147 (33.6%)</td>
</tr>
<tr>
<td>DI</td>
<td>169 (49.5%)</td>
<td>209 (47.8%)</td>
</tr>
<tr>
<td>II</td>
<td>60 (17.5%)</td>
<td>81 (18.6%)</td>
</tr>
</tbody>
</table>
the previously dilated coronary artery segment(s) failed to reveal an association between ACE genotype and phenotype, regardless of whether an additive (DD versus DI versus II; analysis for trend), dominant (DD and DI versus II), or recessive (DD versus DI and II) mode of inheritance was assumed (data not shown).

Likewise, logistic regression analysis with adjustment for several covariates, such as age, gender, BMI, blood pressure, total serum cholesterol, smoking habit, presence of diabetes mellitus, angiographic and occlusion characteristics, and family history of MI, failed to demonstrate a correlation between genotype and clinical outcome (data not shown).

Finally, linear regression analysis, adjusted for the same covariates, showed no significant correlation between genotype and the degree of loss of lumen at 6-month follow-up examination (Table 3).

**Table 3. Quantitative Angiographic Findings**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DD (n=293)</th>
<th>DI (n=422)</th>
<th>II (n=162)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference diameter, mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before angioplasty</td>
<td>2.80±0.53</td>
<td>2.80±0.51</td>
<td>2.84±0.50</td>
<td>0.80</td>
</tr>
<tr>
<td>After angioplasty</td>
<td>2.79±0.52</td>
<td>2.78±0.51</td>
<td>2.82±0.50</td>
<td>0.85</td>
</tr>
<tr>
<td>Follow-up</td>
<td>2.75±0.55</td>
<td>2.74±0.50</td>
<td>2.76±0.48</td>
<td>0.35</td>
</tr>
<tr>
<td>MLD, mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before angioplasty</td>
<td>0.51±0.34</td>
<td>0.50±0.32</td>
<td>0.49±0.31</td>
<td>0.90</td>
</tr>
<tr>
<td>After angioplasty</td>
<td>2.07±0.59</td>
<td>2.06±0.57</td>
<td>2.13±0.54</td>
<td>0.25</td>
</tr>
<tr>
<td>Follow-up</td>
<td>1.42±0.78</td>
<td>1.37±0.80</td>
<td>1.50±0.82</td>
<td>0.80</td>
</tr>
<tr>
<td>Diameter stenosis, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before angioplasty</td>
<td>81.64±11.50</td>
<td>82.16±11.34</td>
<td>82.66±10.77</td>
<td>0.80</td>
</tr>
<tr>
<td>After angioplasty</td>
<td>26.45±12.49</td>
<td>26.63±11.93</td>
<td>24.94±11.03</td>
<td>0.10</td>
</tr>
<tr>
<td>Follow-up</td>
<td>48.47±25.34</td>
<td>50.56±26.32</td>
<td>46.70±26.97</td>
<td>0.45</td>
</tr>
<tr>
<td>Acute gain, mm</td>
<td>1.56±0.65</td>
<td>1.56±0.61</td>
<td>1.65±0.61</td>
<td>0.27</td>
</tr>
<tr>
<td>Late loss, mm</td>
<td>0.66±0.73</td>
<td>0.66±0.76</td>
<td>0.63±0.81</td>
<td>0.35</td>
</tr>
<tr>
<td>Net gain, mm</td>
<td>0.90±0.82</td>
<td>0.90±0.80</td>
<td>1.02±0.86</td>
<td>0.65</td>
</tr>
<tr>
<td>Loss index</td>
<td>0.44±0.61</td>
<td>0.47±0.81</td>
<td>0.38±0.53</td>
<td>0.66</td>
</tr>
</tbody>
</table>

MLD indicates minimal luminal diameter; acute gain, obstruction diameter after angioplasty minus obstruction diameter before angioplasty; late loss, obstruction diameter after angioplasty minus obstruction diameter at follow-up; and net gain, acute gain minus late loss (loss index as the ratio of late loss to acute gain).

Data are mean (±SD).

Discussion

This study represents, to our knowledge, the largest sample so far examined for a possible association of the ACE D/I polymorphism with the outcome after PTCA. A substantial number of preexisting publications had investigated this question, with inconsistent results. Our results support those earlier studies that failed to find such an association.

The initial report of an association of the ACE D/I polymorphism and restenosis after angioplasty was based on a rather small sample of only 82 Japanese patients.5 These findings were not reconfirmed by subsequent, somewhat larger studies,11–13 although others reported results more in line with the original publication.6–10 A review of these studies failed to disclose clear reasons for the discordant results, although in several of these studies allele frequencies showed substantial deviations from those encountered in independent, larger samples from the same ethnic background. Because of the limited size of all these studies, the actual contribution, if any, of the ACE D/I marker to the risk to developing post-PTCA restenosis remains difficult to assess.

A somewhat compelling line of reasoning has frequently been advanced to interpret and support an association of the ACE D allele with increased incidence of restenosis, as observed by some: There is little doubt, based on the consistent results from a large number of investigations in a diverse ethnic groups, that the D allele is associated with significantly higher plasma converting enzyme levels and activities than the I allele, and there is somewhat less well-established evidence that the same is true for tissular enzyme activity, at least in the heart. Considering the well-documented growth-stimulating effects of Angiotensin II, carriers of the DD genotype would, therefore, the argument goes, be more prone to proliferative vascular lesions, as post-PTCA restenosis has commonly been regarded. Further support for the notion that the renin-angiotensin system may play a pivotal role in restenosis is based on experimental models of vascular injury in which ACE inhibitors had been found effective in retarding, preventing, or reversing restenosis.18 However, we have since come to understand that the intimal hyperplasia characteristic of these models (to which smooth muscle cell proliferation appears to contribute importantly) differs markedly from the pathophysiology characteristic of post-PTCA restenosis in humans: In the latter, elastic recoil and smooth muscle cell migration rather than proliferation appear to be the main mechanisms responsible for recur-
rent lumen loss. The dramatic failure of 2 large-scale, randomized trials aimed at demonstrating beneficial effects of ACE inhibition on the occurrence of post-PTCA restenosis is consistent with this view and indicates that growth-promoting actions of the renin-angiotensin system are probably of little consequence to the natural history of post-PTCA restenosis. This much more tangible evidence renders the above-described reasoning for an association of the ACE genotype with restenosis much less compelling. Intriguingly, this may differ from the present situation in in-stent restenosis, a clinical problem of increasing magnitude. Smooth muscle cell proliferation has been reported to be a distinctly more prominent feature in in-stent as compared with post-PTCA restenosis, and the ACE D/I polymorphism has been found associated with the former but not the latter by the same group of investigators. This observation highlights the need for careful accounting of a large variety of nongenetic variables—many of them likely much less obvious than the difference between stented and nonstented coronary revascularization—that may critically affect the results of such association studies.

It is always critical for their proper interpretation of “negative” studies to measure the power they provide to reject the alternate hypothesis. Given the number of cases and control subjects and the respective allele frequencies found, the current study provides 80% power to detect, at an α-error of 0.05, a risk ratio of 1.38 associated with the D/D allele compared with post-PTCA restenosis,22–24 is consistent with this view and indicates that growth-promoting actions of the renin-angiotensin system are probably of little consequence to the natural history of post-PTCA restenosis. This much more tangible evidence renders the above-described reasoning for an association of the ACE genotype with restenosis much less compelling. Intriguingly, this may differ from the present situation in in-stent restenosis, a clinical problem of increasing magnitude. Smooth muscle cell proliferation has been reported to be a distinctly more prominent feature in in-stent as compared with post-PTCA restenosis, and the ACE D/I polymorphism has been found associated with the former but not the latter by the same group of investigators. This observation highlights the need for careful accounting of a large variety of nongenetic variables—many of them likely much less obvious than the difference between stented and nonstented coronary revascularization—that may critically affect the results of such association studies.

Conclusions
This large, prospective study provides good power to exclude a clinically significant association of ACE D/I allele carrier status with the risk of developing post-PTCA restenosis in white individuals. The present data do not address the possible association of the polymorphism with in-stent restenosis and cannot exclude the possibility that other mutations in the ACE gene may contribute to the risk of post-PTCA restenosis. Our results indicate, however, that the ACE D/I polymorphism is not a useful marker for assessing the risk of restenosis after coronary angioplasty.

Acknowledgment
This work was supported by a Research Development Award (K04-HL-03138-01) from the National Heart, Lung, and Blood Institute to Dr Klaus Lindpaintner.

References
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Hypertension. 2001;37:851-855
doi: 10.1161/01.HYP.37.3.851

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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