Angiotensin-Converting Enzyme DD Genotype and Cardiovascular Disease in Heterozygous Familial Hypercholesterolemia

Jean P. O’Malley, BS; Cheryl L. Maslen, PhD; D. Roger Illingworth, MD, PhD

Background—Controversy exists as to whether the deletion/deletion genotype (DD) of the ACE gene polymorphism increases the risk of myocardial infarction (MI). Studies have suggested that the ACE DD genotype is associated with increased plaque instability. We hypothesized that the ACE DD genotype may increase the risk of myocardial infarction and coronary heart disease (CHD) in patients with heterozygous familial hypercholesterolemia (FH) or familial defective apolipoprotein B-100 (FDB) who, as a group, are at high risk of having lipid-rich plaques in their coronary arteries.

Methods and Results—We determined the ACE genotypes and incidence of MI or surgical intervention for CHD in 213 adult patients with heterozygous FH or FDB. The incidence of MI in 35 male patients who carried the ACE DD genotype was 2.5 times that observed in male patients with the II or DI genotypes, and the incidence of CHD in male patients with the DD genotype was 2.2 times higher than in those who had ACE DI. The potential effects of ACE genotype on CHD could not be directly compared in female patients because of a disparity in the smoking history of the genotypic groups. From logistic regression analysis, the estimated odds ratio associated with the ACE DD genotype was 2.57 for MI and 2.21 for CHD adjusted for age, sex, and smoking history.

Conclusions—The ACE DD genotype is associated with an increased risk of MI and CHD in patients with heterozygous FH or FDB. Determination of the ACE genotype in asymptomatic FH and FDB patients provides an additional means to identify those patients at greatest risk for the premature development of CHD. (Circulation. 1998;97:1780-1783.)

Key Words: angiotensin ■ myocardial infarction ■ hypercholesterolemia ■ coronary disease

The ACE I/D polymorphism was identified in 1990 and has been shown to be strongly associated with variations in the levels of ACE in plasma, with 47% of the observed phenotypic variation being attributable to the segregation of the ACE I/D alleles. The ACE DD genotype has been shown to be associated with increased plasma concentrations of circulating ACE, which results in the enhanced conversion of angiotensin I to II; angiotensin II has been shown to increase the influx of 125I-labeled LDL into the arterial wall of rabbits. Interest in the effects of the ACE polymorphism on cardiovascular disease was stimulated by results of a large multicenter case-control study in which the presence of the ACE DD genotype was associated with an overall 1.3-fold increased risk of MI, with an odds ratio of 3.2 in a subset of men considered at low risk for CHD. Although some subsequent studies confirmed the higher frequencies of the ACE DD genotype in patients with MI or CHD, the finding has not been consistently observed, and the inconsistency has led to confusion. The ACE DD genotype has been shown to be associated with intimal-medial thickening in the carotid artery, and a 2-fold-higher adjusted odds ratio for restenosis after intracoronary stent implantation. In a recent meta-analysis of 15 studies involving 3394 MI patients and 5479 control subjects, the odds ratio for MI in patients with the DD genotype versus the DI genotype was 1.26. It has been proposed that plaque instability may be increased in patients with the ACE DD genotype, thereby putting these patients at increased risk for plaque rupture, unstable angina, and MI.

Patients with heterozygous FH or FDB are known to be at substantially increased risk for premature MI and CHD because of their high circulating levels of LDL. These patients are particularly prone to the development of lipid-rich plaques in their coronary arteries and may be particularly susceptible to arterial stenosis requiring surgical intervention in the presence of ACE DD genotype–associated intimal-medial thickening or to plaque rupture in the presence of ACE DD genotype–associated plaque instability. We hypothesized that patients with heterozygous FH or FDB who also carry the ACE DD genotype should be at higher risk for MI and surgical intervention for CHD than patients with the ACE DI genotype.
**Methods**

Blood samples were obtained from patients with heterozygous FH or FDB (96 men and 117 women; age, ≥40 years) attending the Lipid Disorders Clinic at Oregon Health Sciences University. Patients were diagnosed to have heterozygous FH or FDB on the basis of persistent primary hypercholesterolemia with elevated concentrations of LDL cholesterol, an inheritance pattern consistent with autosomal dominant, and the presence of tendon xanthomas in the index patient or in a first-degree relative. Heterozygous FH and FDB are both autosomal-dominant genetic disorders that are distinguishable at the molecular level but result in a uniform phenotype of highly elevated plasma concentrations of LDL. All patients underwent a comprehensive history and physical examination and blood samples were obtained for Lp analysis and the exclusion of secondary causes of hyperlipidemia. Clinical characteristics of the patients, including previous MI or history of angioplasty or coronary artery bypass graft surgery, were based on patient history and examination and review of medical records. Patients whose medical records disclosed a history of diabetes mellitus were excluded from this study.

Genomic DNA was isolated by standard methodology, and ACE genotypes were determined by polymerase chain reaction analysis according to the method of Rigat et al., modified by the inclusion of 5% (vol/vol) dimethyl sulfoxide in the final reaction mixture to aid in the amplification of the I allele.

CHD was defined as a history of MI or surgical intervention for CHD. Hypertension was defined by a history of having been prescribed hypotensive medication. No differences were found in the incidence of CHD or MI between patients with the ID and II genotypes, and patients from these two genotypic groups have been combined into one group designated II+DI for statistical analysis and comparison of the incidence of cardiovascular disease. Statistical analysis of the data was performed by contingency testing. Odds ratios were computed from a multivariate logistic regression model to provide an estimate of the relative risk of MI or CHD. The model tested the association of the presence or absence of the ACE DD genotype with MI or CHD risk, with adjustment for the effects of sex and smoking history. Additional analyses were performed using apo E genotype, hypertension, Lp(a) concentrations, or untreated LDL cholesterol levels in those patients in whom complete information was available. Multivariate logistic regression analyses were done with a JMP statistical software package, version 3.15 (SAS Institute Inc).

The 213 patients with heterozygous FH or FDB consisted of 163 unrelated subjects and 50 patients who were derived from 20 separate families. In 5 of the families, more than one patient had CHD, but only 1 family included 2 patients with the ACE DD genotype who concurrently had CHD. Of the patients with heterozygous FH, 2 were of Japanese ancestry, 1 was of Chinese ancestry, and the remaining 210 were Caucasian.

**Results**

The frequencies of the ACE II, DI, and DD genotypes in the 213 patients with heterozygous FH or FDB (21%, 46%, and 33%, respectively) were similar to those predicted for Hardy Weinberg equilibrium (19%, 49%, and 32%, respectively), indicating that we had not received a disproportionate referral of patients enriched or depleted in one genotypic group. The distribution of known cardiovascular risk factors and Lp values in male and female patients with the ACE II+DI and DD genotypes is shown in Table 1; with the exception of smoking in women, the groups are comparable.

The incidence of CHD and previous MI in male and female patients classified according to their ACE genotypes is shown in Table 2. Male patients with heterozygous FH or FDB were 2.5 times more likely to have suffered an MI if they carried the ACE DD genotype than similar patients with the ACE

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**TABLE 1. Cardiovascular Risk Factors of Heterozygous FH and FDB Patients by ACE Genotype**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Men</th>
<th>Women</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>II+DI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>61</td>
<td>84</td>
<td>35</td>
<td>33</td>
</tr>
<tr>
<td>Age, y</td>
<td>57±9</td>
<td>54±9</td>
<td>56±11</td>
<td>55±9</td>
</tr>
<tr>
<td>Prior history of smoking, n (%)</td>
<td>31 (51)</td>
<td>40 (48)</td>
<td>16 (46)</td>
<td>9 (26)</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>135±24</td>
<td>131±19</td>
<td>130±15</td>
<td>127±20</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>78±13</td>
<td>78±10</td>
<td>80±7</td>
<td>77±14</td>
</tr>
<tr>
<td>History of hypotensive therapy, n (%) (number of patients)</td>
<td>58 (23)</td>
<td>82 (34)</td>
<td>35 (23)</td>
<td>30 (17)</td>
</tr>
<tr>
<td>LDL cholesterol (untreated levels), mg/dL</td>
<td>315±63</td>
<td>298±58</td>
<td>308±57</td>
<td>321±54</td>
</tr>
<tr>
<td>No. of patients</td>
<td>47</td>
<td>75</td>
<td>27</td>
<td>28</td>
</tr>
<tr>
<td>Age hypolipidemic therapy started, y</td>
<td>44±9</td>
<td>43±11</td>
<td>46±6</td>
<td>41±11</td>
</tr>
<tr>
<td>No. of patients</td>
<td>57</td>
<td>82</td>
<td>35</td>
<td>31</td>
</tr>
<tr>
<td>Lp(a) levels, mg/dL</td>
<td>31±31</td>
<td>31±26</td>
<td>31±26</td>
<td>33±33</td>
</tr>
<tr>
<td>No. of patients</td>
<td>49</td>
<td>71</td>
<td>31</td>
<td>25</td>
</tr>
<tr>
<td>Apo E genotype (E4/4 or E4/3), %</td>
<td>30</td>
<td>26</td>
<td>17</td>
<td>30</td>
</tr>
<tr>
<td>Apo E genotype (E2/E3), %</td>
<td>13</td>
<td>12</td>
<td>20</td>
<td>23</td>
</tr>
<tr>
<td>No. of patients</td>
<td>54</td>
<td>78</td>
<td>35</td>
<td>30</td>
</tr>
</tbody>
</table>
DI+II genotypes (40% versus 16% incidence, respectively; \(P<.02\)). The frequency of documented CHD (defined by previous coronary artery bypass surgery, angioplasty, or MI) was also found to be 2.2 times higher in male patients with the ACE DD genotype (71% incidence in ACE DD males) compared with male patients with the ACE DI+II genotype, in whom the overall incidence was 33% \( (P<.001)\). In contrast to the findings in male patients with heterozygous FH or FDB, we observed no significant differences in the incidence of either MI or CHD in female patients with the ACE DD genotype compared with female patients with the DI+II genotypes (Table 2). However, the incidence of MI and CHD was significantly lower than in their male counterparts (Table 2), and a prior history of cigarette smoking was significantly less common in female patients with the ACE DD genotype than in the other genotypic groups (Table 1).

Multiple logistic regression analysis of the data indicates that, although male gender and a positive history of cigarette smoking are each independently associated with an increased risk of MI and CHD of more than 3-fold, the ACE DD genotype is itself associated with a 2.57-fold increase in the risk of MI \( (P=.018)\) and a 2.21-fold increase in the risk of CHD \( (P=.021)\) after adjustment for the effects of sex and smoking history (Table 3). Similar analyses were run on subsets of the FH and FDB patients testing for the effect of apo E genotype, Lp(a) concentrations, hypertension, untreated LDL cholesterol levels, and the age at which hypolipidemic therapy was started. In each case, the tests were run on all patients for whom the additional information was available \( [n=197, 176, 205, \text{and } 205 \text{ for apo E genotype, Lp(a), hypertension, and the age at start of hypolipidemic therapy, respectively}]\). No significant association was observed between any of these variables and the risk of MI and CHD in these patients. The ACE DD genotype-associated odds ratios for MI and CHD were unaffected by adjustment for these variables. Information on untreated LDL cholesterol levels was not available in 12 patients with a history of a previous MI, and of these, 7 carried the ACE DD genotype. The effects of this variable could not be tested by multiple logistic regression analysis; however, untreated LDL cholesterol levels did not differ significantly between ACE genotypic groups in the 177 patients for whom this information was available (Table 1), making it unlikely that the increased incidence of MI and CHD observed in the FH and FDB patients who carry the ACE DD genotype was due to differences in the baseline concentrations of LDL cholesterol.

**Discussion**

We found that the presence of the ACE DD genotype is associated with a significant increase in the risk of patients with heterozygous FH or FDB suffering a MI or requiring surgical intervention for coronary artery disease. The adjusted odds ratio for the ACE DD genotype associated risk of MI (2.5) is higher than the mean value reported in a recent meta-analysis of 15 previous studies (none of which focused on patients with primary hypercholesterolemia), in which the odds ratio for MI in patients with the DD genotype versus those with a DI+II genotype was 1.26.\(^{14}\) Our results are consistent with the hypothesis that the presence of the ACE DD genotype may accelerate the risk of suffering a cardiovascular event caused by plaque rupture in those patients who are at high risk of having unstable lipid-rich plaques in their coronary arteries.\(^{19,24}\) Potential mechanisms by which the presence of the ACE DD genotype may increase the risk of plaque rupture in patients with heterozygous FH include accelerating the rate of influx of LDL particles into the arterial wall with a resultant increase in plaque lipid content,\(^{19}\) promoting an increase in local shear forces on the arterial wall,\(^{24}\) and promoting endothelial dysfunction and vascular smooth muscle cell proliferation.\(^{13,25}\)

Although our results did not disclose an increased incidence of MI or CHD in female patients with heterozygous FH with the ACE DD genotype compared with female patients with the DI+II genotypes, the overall incidence of CHD in the female patients was lower, and the female patients who carried the ACE DD genotype had a lower incidence of cigarette smoking than ACE DI+II female patients (Table 1) and consequently had a decreased risk of developing coronary disease. The adjusted odds ratios estimate that the ACE DD genotype is associated with a 2.57-fold increased risk of MI and a 2.21-fold increased risk of CHD after adjustment for the effects of gender and smoking history. Our results indicate that the determination of ACE genotypes in patients with heterozygous FH or FDB may be useful as an additional

**TABLE 2. Effect of ACE Genotype on the Incidence of CHD in Patients With Heterozygous FH and FDB**

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>CHD, n (%)</th>
<th>MI, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE DD</td>
<td>20 (33)</td>
<td>10 (16)</td>
</tr>
<tr>
<td>ACE DI</td>
<td>24 (71)†</td>
<td>14 (40)*</td>
</tr>
</tbody>
</table>

*P<.02; †P<.01.

**TABLE 3. Adjusted Odds Ratios for Cardiovascular Risk Factors in Patients With Heterozygous FH and FDB**

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>MI 95% Confidence Intervals</th>
<th>P</th>
<th>CHD 95% Confidence Intervals</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE DD genotype</td>
<td>2.57 1.17–5.67</td>
<td>.018</td>
<td>2.21 1.47–2.21</td>
<td>.021</td>
</tr>
<tr>
<td>Prior cigarette smoking</td>
<td>3.19 1.47–7.29</td>
<td>.004</td>
<td>3.71 1.97–7.21</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

The ACE DD genotype odds ratios for MI and CHD were not affected by adjustment for apo E genotype, Lp(a) concentrations, hypertension, or the age at which hypolipidemic therapy was started. \(n=213\).
discriminator of cardiovascular risk in asymptomatic patients. More aggressive treatment would seem appropriate for those patients with the ACE DD genotype; these recommendations parallel those previously suggested for patients with heterozygous FH who have concurrently increased plasma concentrations of Lp(a).  

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