Angiotensin-Converting Enzyme Genotypes and Risk for Myocardial Infarction in Women

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Objectives. We tested for an association between the angiotensin-converting enzyme (ACE) DD polymorphic genotype and myocardial infarction (MI) in a sample group composed exclusively of women.

Background. The human ACE gene occurs with either an insertion (I allele) or a deletion (D allele) of a 287-base pair (bp) Alu element. Part of the variance in serum ACE levels may be accounted for by this polymorphism. Also, the DD genotype has been associated with an increased risk of MI in predominantly male populations. However, the risk in women is poorly defined.

Methods. Genomic DNA was extracted from buffy coat blood using a phenol/chloroform method. Angiotensin-converting enzyme alleles were identified using primers to bracket the insertion region in intron 16. Amplification using polymerase chain reaction allowed identification of a 490-bp (I allele) or a 190-bp (D allele) product, or both.

Results. Allelic and genotypic frequencies in control subjects were similar to those reported in mostly male populations, and frequencies of genotypes were in the Hardy-Weinberg equilibrium. In contrast, the distribution of genotypes in patients with MI diverged from the equilibrium. Specifically, DD genotypic frequency was increased in women with (n = 141) versus without (n = 338) a previous MI (39% vs. 29%, odds ratio [OR] 1.54, 95% confidence interval 1.02 to 2.32, p < 0.04). Risk was particularly increased in women <60 years old (OR 2.04, p < 0.05). In contrast, the DD genotype did not predict angiographic coronary artery disease.

Conclusions. Consistent with findings in male-dominated populations, a modest association of the ACE DD genotype with MI was found in women. The basis for this association requires further study.

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women. Moreover, the incidence, timing and known risk factors for MI show differences in women as compared with men (22, 23). Rates of MI lag by a decade in women as compared with men and show gender-related distinctions by hormonal and diabetic status and lipid profile. Genetic risk factors also might differ qualitatively and quantitatively by gender. Thus, we sought to evaluate the association of the ACE polymorphism with MI risk in a sample group composed exclusively of women.

### Methods

**Study hypotheses.** We prospectively postulated that, as with men, women with the homozygous ACE deletion genotype (DD) would be at greater risk of MI than those without this genotype (recessive model) (21). Also, we tested whether MI was significantly associated with the presence of the D allele (13).

**Study group.** The study group consisted of two subgroups. The first, or pilot, subgroup (n = 295) consisted of a subset of a previous study of a mixed-gender group (13) prospectively studied for an overall association of the I/D polymorphism with MI. In this group there was a trend toward an association of the D allele (28%) with women who had a history of MI (n = 105) to provide adequate power to detect an OR for MI of ~1.5 for DD versus non-DD (ID + II) genotypes.

**Patient characteristics.** All patients were women of unrestricted age who presented for angiography at LDS Hospital because of either symptoms relating to suspected CAD or unrelated conditions requiring angiographic evaluation (e.g., valvular disease, cardiomyopathy). The patients gave written informed consent for a blood draw for use in confidential deoxyribonucleic acid (DNA) bank studies (13, 24). The patients were drawn from a population primarily of Northern European (Anglo-Scandinavian) descent (Utah, southwestern Idaho and southeastern Wyoming) that is ethnically and genetically representative of U.S. whites (25).

Key demographic characteristics for the patients were captured on computerized angiographic data forms, including age, gender and history of MI (24). Angiographic assessment of CAD was determined by a review of angiograms by the patient's cardiologist and entered into the computer database in a format modified after the Coronary Artery Surgery Study (CASS) protocol (24, 26). Patients were designated as having CAD if they had >60% stenosis in at least one coronary artery or its major branch and no CAD if <10% stenosis was present. Other patients with minor CAD (10% to 60% stenosis) were given an “indeterminate” CAD status and were not used in genotype by CAD analyses. Final designations of MI and CAD status were made after considering arteriographic and ventriculographic results, together with patient history, without knowledge of DNA genotype. The control group included angiographically studied patients without acute or old MI regardless of CHD status.

**DNA genotyping.** Peripheral blood was collected in EDTA, and the DNA was extracted from the leukocyte buffy coat by phenol/chloroform extraction and alcohol precipitation as previously described (27). The ACE alleles were identified by the amplified fragment length polymorphism method. This method has been described elsewhere (7). Briefly, primers that bracket the insertion region in intron 16 were used in the polymerase chain reaction to produce either an amplified 408-bp (I allele) or a 190-bp (D allele) product, or both. The upstream primer (5’ to 3’) was: CTGGAGACCCTCCATCCTTTC. The downstream primer (5’ to 3’) was: GATGTTGCCATCACATTGTCGAT. Amplification was for 30 cycles; each cycle consisted of a denaturation segment at 94°C for 1 min, an annealing segment at 62°C for 45 s and an extension segment at 72°C for 1 min. A final extension segment at 72°C for 5 min was included after the final cycle. All suspected deletion mutants were reamplified using the downstream primer and a third primer (5’ to 3’: TTTGAGACG-GAGTCTCCTGTC); this combination produces a 408-bp product for the insertion allele and no product for the deletion allele (28). The products were visualized by electrophoresis through 1.5% agarose gel, followed by staining with 1 μg/ml of ethidium bromide in tris-borate-EDTA buffer. The genotype was identified by an experienced observer who had no knowledge of the patients’ clinical characteristics (Fig. 1).

**Statistics.** Comparisons between genotype frequencies were done using chi-square analysis; ORs with 95% CIs were calculated as previously described (29). No significant difference in the frequency of the DD genotype among patients with MI was observed between the pilot and expanded patient groups (p = 0.46), so they were combined and considered together as a single series in the presentation of the results.

A two-tailed p value ≤0.05 was considered significant for
the primary hypothesis (risk of MI in women with the DD vs. non-DD genotype). P values for secondary hypothesis testing were not corrected for multiplicity of comparisons and should be viewed with caution.

Results

Patient groups. A total of 490 women with angiographically defined CAD were studied; MI status was known for 479 of them (141 had a history MI and 338 did not). CAD was severe in 250 patients, absent/minimal in 225 and intermediate (10% to 60% stenosis) in 15, who were excluded (see Methods). Thus, 475 patients with either advanced or negligible CAD and 479 with or without a known history of MI formed the basis for the analysis. Patient characteristics are summarized in Table 1. As expected, patients with CAD were older, were more frequently hypertensive, diabetic and hyperlipidemic and more often tended to be smokers than those without CAD. Common risk factors, including age, smoking and diabetes (trend), were more prevalent in patients with MI than in control subjects.

Genotypic frequencies. Genotypic and D allelic frequencies for the study groups are shown in Table 2. The control group’s genotypic frequencies are in agreement with the frequencies predicted by the Hardy-Weinberg equilibrium. The D allelic frequency in the control groups without CAD and without MI was 52% to 53%. These allelic and genotypic frequencies also are similar to those previously reported in a large European control population (9,30), as well as in a smaller sample of control subjects from the western United States (31) and in our previously reported, angiographically assessed group from Utah (13). In contrast to control subjects, the genotypic distribution in women with MI (but not CAD) differed from Hardy-Weinberg expectations (chi-square statistic 4.6, p = 0.03).

Association between ACE I/D polymorphism and MI. A significant increase in the frequency of the DD genotype versus non-DD genotypes was found among women who had a previous MI (n = 141) compared with control subjects without MI (with or without CAD; n = 338)—39.0% versus 29.3%, OR 1.54, 95% CI 1.02 to 2.32, p = 0.038 (Table 3, Fig. 2). Overall (binomial) distributions of genotypes tended to differ between MI and non-MI groups (Table 2), although these differences did not achieve significance (p = 0.11).

We also tested relative frequencies of the D allele in

<p>| Table 1. Characteristics of Patients With Disease and Control Subjects* |
|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>Patients With MI (n = 141)</th>
<th>Control Group (no MI) (n = 338)</th>
<th>Patients With CAD (n = 250)</th>
<th>Control Group (no CAD) (n = 225)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>64 ± 10†</td>
<td>60 ± 11</td>
<td>64 ± 10†</td>
<td>58 ± 11</td>
</tr>
<tr>
<td>Range</td>
<td>48–89</td>
<td>17–84</td>
<td>28–89</td>
<td>17–81</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>138 ± 22</td>
<td>133 ± 23</td>
<td>138 ± 25†</td>
<td>129 ± 19</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>76 ± 12</td>
<td>74 ± 11</td>
<td>74 ± 12</td>
<td>76 ± 11</td>
</tr>
<tr>
<td>Smoker</td>
<td>35†</td>
<td>20</td>
<td>26†</td>
<td>21</td>
</tr>
<tr>
<td>Diabetes</td>
<td>22</td>
<td>16</td>
<td>25†</td>
<td>10</td>
</tr>
<tr>
<td>Hypercholesterolemia (% 220 mg/dl)</td>
<td>40</td>
<td>44</td>
<td>46†</td>
<td>28</td>
</tr>
</tbody>
</table>

*Based on 95% complete data for patients with myocardial infarction and 98% for patients with coronary artery disease, except for cholesterol (data from subset of 127), †p ≤ 0.05, ‡p ≤ 0.001 versus control subjects. Data presented are mean value ± SD or percent of patients. CAD = coronary artery disease; MI = myocardial infarction.
patients and control subjects (Table 2). D allelic frequency tended to be enriched in patients with MI (59%) compared with those without MI (53%), although the difference did not achieve significance (p = 0.11).

In contrast to DD, ID did not confer increased risk for MI (OR 0.85 for ID vs. II, p = 0.53). Frequencies of the II genotype were similar in patients and control subjects (21% vs. 23%), whereas the D allele tended to occur in combination with the I allele somewhat more frequently in control subjects than in patients (48% vs. 40%, p = 0.11).

We also tested a dominant model that compared risk of disease (MI) of the DD + ID versus II genotypes; a significant difference was not found (OR 1.11, p = 0.67). Finally, a homozygous comparison (DD vs. II) was made; a trend toward an increased risk of DD was found (OR 1.44), although it did not achieve significance (p = 0.18), perhaps because of reduced power after excluding subjects with the ID polymorphism.

ACE polymorphism associations with MI by age. The increased risk of MI associated with the DD polymorphism was especially evident in younger women (<60 years old, n = 187), who showed an OR of 2.04 for MI (95% CI 1.00 to 4.16, p = 0.048) when DD and non-DD genotypes were compared (Table 3, Fig. 2). For older women (≥60 years old, n = 292), the OR was 1.32 (CI 0.79 to 2.2, p = 0.28) (Fig. 2). Overall (binomial) distributions of the three I/D genotypes also differed significantly from those of age-comparable control groups in younger women (p = 0.037) but not in older women (p = 0.45) (Fig. 3). In contrast to age, smoking status, diabetes and hypertension status did not tend to importantly influence the relative risk of the ACE DD genotype for MI.

Association between ACE I/D polymorphism and CAD. Consistent with the findings in our male-dominated series (13), only small differences in DD genotypic and in D allelic frequencies that were not significant were found in women with angiographically documented CAD compared with those without CAD (Table 2).

Discussion

Summary of study results. In this study of moderate size (n = 490) in women with angiographic assessment of CAD, we found a statistically significant relation of moderate degree between the DD genotype and risk of MI (OR 1.54). These results are similar to those in our angiographically assessed series of men (13) and in another meta-analysis (21). In an exploratory subgroup analysis, an increased relative risk of MI with the DD genotype was especially apparent in younger women.

Comparison with previous studies. In our previous study of 402 men (age range 30 to 64 years), the occurrence of MI was significantly associated with the DD genotype, which was present in 36% of patients with CAD plus MI and 25% of control subjects with CAD and without MI (OR 1.63, p = 0.02). The relative risk for MI of the DD genotype is thus quantitatively similar in women and in men in our group and may be even greater in younger women (Fig. 2).

A recent meta-analysis (15 studies) of the association between the ACE I/D polymorphism and MI in male-dominated populations found a mean OR of 1.26 for MI of the DD versus ID + II genotypes (95% CI 1.15 to 1.39, p < 0.001) (21). The distributions of genotypes in the control subjects

Figure 2. Odds ratios (diamonds) with 95% CIs (horizontal lines) for DD versus non-DD genotypes among women (total group and according to age <60 and ≥60 years) and men (13) who were angiographically assessed.

Figure 3. Genotypic frequencies by age for patients with MI and control subjects without MI. Distributions are significantly different for patients with versus without MI who were <60 (p < 0.04) but not ≥60 years old (p = 0.45).

Table 3. DD and Non-DD Genotype Frequencies in Women by Age and Myocardial Infarction Status

<table>
<thead>
<tr>
<th></th>
<th>DD</th>
<th>Non-DD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All women</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MI (n = 141)</td>
<td>39.0%†</td>
<td>61.0%</td>
</tr>
<tr>
<td>No MI (n = 338)</td>
<td>29.3%</td>
<td>70.7%</td>
</tr>
<tr>
<td>Women &lt; 60 yr old</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MI (n = 42)</td>
<td>42.9%†</td>
<td>57.1%</td>
</tr>
<tr>
<td>No MI (n = 145)</td>
<td>26.9%</td>
<td>73.1%</td>
</tr>
<tr>
<td>Women ≥ 60 yr old</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MI (n = 99)</td>
<td>37.4%</td>
<td>62.6%</td>
</tr>
<tr>
<td>No MI (n = 193)</td>
<td>31.1%</td>
<td>68.9%</td>
</tr>
</tbody>
</table>

*Non-DD = ID + II. †p < 0.05 versus control group (no MI). Data are presented as percentage of patients. Abbreviations as in Tables 1 and 2.
were 22.7% for II, 49.0% for ID and 28.3% for DD, virtually identical to those of our female control subjects (Table 2).

In contrast to most previous studies, Schuster et al. (32) reported results separately by gender. Also, uniquely, they found an association between the ACE I/D polymorphism and MI for women but not for men. For the entire group (n = 390), a significant association between the homozygous (DD) genotype and MI was observed (relative risk 1.59, 95% CI 1.03 to 2.48). When the group was subclassified by gender, the results were significant for women only (n = 90, 25 with MI): 48% of women with MI versus 23% of control subjects had the DD genotype. However, the possibility that this apparent dichotomous behavior according to gender was due to chance in their relatively small study (especially with respect to women) was not excluded. Indeed, the study has too few female patients with MI to provide a reliable risk estimate of the I/D polymorphism by gender.

We did not find the intermediate-risk association between MI and the ID genotype in women that has been reported in some (but not all) male-dominated series (21). Schuster et al. (32) also failed to observe an intermediate risk with the ID genotype. Indeed, the ID genotype was more frequent among women without MI (58%) than with MI (40%), whereas II genotypic frequencies were similar in patients and control subjects.

Given the known differences in some risk factors for MI by gender (22,23), a real difference in the direction or magnitude of the risk association in women with the ID genotype is possible. However, we do not know of a compelling reason to expect such a difference. Thus, the association (if any) between the heterozygous (ID) genotype and MI risk in women should be reexamined in other groups.

As in our study of men (13), we found little evidence for a link between the I/D polymorphism and the pathophysiologic steps leading to the development of atherosclerosis, as manifested by angiographically defined coronary artery stenoses. Rather, the link was observed with the transition from CAD to MI (virtually all patients with MI also had CAD), suggesting that the mechanisms associated with this transition should be explored.

Pathophysiologic considerations. By its pivotal position in controlling activity of the renin-angiotensin system, ACE may play an important role in various aspects of cardiovascular pathophysiology, including acute MI (1–5,33). Increased rates of angiotensin II generation may lead to increases in neointimal proliferation, particularly after endothelial injury, and vascular tone, with a propensity to vasospasm. Similarly, the vasodilatory effects of bradykinin may be disturbed by accelerating its degradation. A prothrombotic state may be favored through a number of intermediary mechanisms (33).

Although the initial report of an association between the ACE I/D polymorphism and MI by Cambien et al. (9) has been followed by confusion generated by discordant reports (20), an overview of data is still consistent with an effect, although of lesser overall magnitude than originally described (21).

That the I/D polymorphism is associated with functional consequences was suggested by studies linking it with control of plasma ACE levels (8,10,34,35). Rigat et al. (8) reported that half of the interindividual variance in serum enzyme levels could be accounted for by the polymorphism and the DD genotype, as compared with the II genotype, was associated with a twofold increase in plasma ACE activity. However, MacKenzie et al. (35) later found that although there was a clear-cut association between the I/D polymorphism and ACE levels (in Jamaicans), another unlinked gene also appeared to be implicated (i.e., the initial estimates of the influence of the I/D polymorphism on serum ACE levels may have been biased upwards). Levels of ACE in cardiac tissue also appear to be influenced by the polymorphism (36). Nevertheless, it is recognized that the D allele in the ACE gene may simply be in linkage disequilibrium with a nearby, pathogenetically relevant mutation (32). Finally, pharmacologic inhibition of ACE activity may prevent myointimal proliferation after vascular injury (37) and, through uncertain mechanisms, has been observed to reduce the risk of recurrent MI in secondary prevention studies (38,39).

The lack of a linear gradient in MI risk for the DD versus ID versus II genotypes suggests a possible recessive model in women, whereby the DD homozygote is necessary to cause a measurable increase in disease through its effects on circulating and tissue ACE levels or other mechanisms. Women might have a higher threshold than men for the effects of the D allele, requiring homozygosity. However, the lack of an association between the ID genotype and intermediate risk of MI also may be due to chance in a study of moderate size and power.

Study limitations. This prospective study still suffers from limitations of cross-sectional, observational studies. The diagnosis of MI relied on historic information rather than prospective follow-up data. Only survivors of MI were studied; theoretically, the DD genotype could mark enhanced survival after MI rather than be a risk factor. A number of observations argue against this possibility, as summarized by Singer et al. (20) and Dakik et al. (40). In our own group, DD frequencies were not enriched with advancing age.

A methodologic issue in earlier studies involved the mistyping of ID as DD; current methodology avoids these earlier pitfalls (28), and any residual mistyping would need to occur differentially in patients and control subjects. Indeed, we undertook retyping in a subset of our groups (~10%) and found identical results; also, our genotypic frequencies were virtually identical to those previously reported in male or mixed-gender groups (9,13,30,31).

The limited number of subjects in some subgroups of interest, eg, low-risk women who are <60 years of age, non-smokers and non-diabetics, prevented meaningful analysis. Formal logistic regression analysis was not performed to adjust odds ratios for baseline factors. We were not able to directly measure plasma ACE levels in our patients; others have related ACE levels with genotype (8,36). Finally, establishment of an association does not demonstrate causality; for example, the D allele may be in linkage disequilibrium with a
mutation in a nearby gene that is actually responsible for promoting MI.

Implications and conclusions. The finding in our study of an association between the ACE DD genotype and increased risk of MI in women (especially younger women) suggests that genetically determined pathophysiologic mechanisms may be in large measure independent of gender, given the observation of an association of similar magnitude in men. However, the determination of MI risk is multifactorial, with many environmental and genetic factors interacting in a complex and as yet incompletely understood way. Thus, it is not surprising that associations between risk and common polymorphisms are moderate at best (41) and have shown varying results in studies among groups with different genetic and environmental backgrounds.

The risk increment of the I/D polymorphism is likely to be too small to be of routine value in individual risk assessment; rather, studies of the polymorphism may contribute more to pathophysiologic and epidemiologic insights in patient groups. Continued observations in larger, well-defined populations, with prospective follow-up and control for other genetic and environmental factors of relevance, are needed and are likely to lead to a better understanding of the genetically determined risk of MI, including the contribution of ACE gene activity to MI.

References


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