Plasma Level and Gene Polymorphism of Angiotensin-Converting Enzyme in Relation to Myocardial Infarction

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Background The angiotensin-converting enzyme (ACE) plays an important role in the production of angiotensin II and the degradation of bradykinin, two peptides involved in cardiovascular homeostasis. Presence of a polymorphism in the ACE gene (ACE Ss) has been postulated from segregation analysis of plasma ACE in families. This putative polymorphism, which strongly affects the plasma and cellular levels of ACE, probably by modulating ACE gene transcription, has not yet been identified at the molecular level; however, an insertion/deletion polymorphism is present in the 16th intron of the ACE gene (ACE I/D) and appears to be a very good marker for ACE Ss. The biological role of ACE suggests that the ACE gene polymorphism could affect the predisposition to myocardial infarction (MI).

Methods and Results We have recently shown, in a large case-control study (ECTIM), that the marker allele D of the ACE gene, which is associated with higher levels of ACE in plasma and cells, was more frequent in male patients with MI than in control subjects, especially in patients considered at low risk. ACE activity has now been measured from frozen aliquots of plasma in a large subsample of the ECTIM study (n=1086). Plasma ACE level did not differ between patients and control subjects in the older age group (≥55 years) but was higher in patients than in control subjects in the younger age group (<55 years); P<.005 after adjustment on ACE I/D and other risk factors. In patients, plasma ACE levels decreased with age (R=-.225, P<10^-4), but in control subjects no such trend was observed. In the low-risk group (ApoB <1.25 mg/dL, body mass index <26 kg/m^2, and not treated with hypolipidemic drugs), plasma ACE level was increased in patients when compared with control subjects among homozygotes and heterozygotes for the ACE I allele (P<.015). Analysis of the distribution of plasma ACE by using a unimodal analysis conditional on the marker genotype ACE I/D enabled us to infer the frequencies and effects of the postulated ACE Ss genotypes. The results suggest that the higher plasma ACE levels in patients than in control subjects in the younger age group were due to a difference in frequency of the postulated S allele (0.47 versus .36).

Conclusions These results extend our previous findings and indicate that plasma ACE level may be a risk factor for MI, independent of the ACE I/D polymorphism. (Circulation. 1994;90:669-676.)

Key Words • myocardial infarction • angiotensin • genes

The angiotensin I-converting enzyme (ACE) is an ectoenzyme of vascular endothelial cells, also secreted in plasma, that plays an important role in cardiovascular homeostasis through angiotensin II formation and bradykinin inactivation.1 In humans, the levels of plasma and cellular ACE are strongly genetically determined.2-5 Family studies suggest that approximately 50% of the interindividual variability of plasma ACE is attributable to a major gene polymorphism, the postulated ACE Ss polymorphism,2,4 which has not yet been characterized at the molecular level. After the ACE gene was cloned,6 an insertion/deletion (ACE I/D) polymorphism resulting from the presence/absence of a 287 base pairs fragment in the 16th intron of the ACE gene was identified.7 The deletion (D) is codominantly associated with the mean immunoreactive level3 and activity4,5 of plasma and cellular ACE, ie, levels are higher in homozygotes for the D allele than in homozygotes for the I allele and are intermediate in heterozygotes. The ACE I/D polymorphism is a marker for the postulated functional variant ACE Ss and is associated with 28% to 44% of the interindividual variability of plasma ACE activity or immunoreactive level.3,4 Recently, in a large multicenter case-control study (ECTIM), an increased risk of myocardial infarction (MI) and an increased frequency of parental MI were demonstrated in subjects carrying the ACE D allele,8,9 in particular in patients conventionally considered low risk.8 These observations suggested that a high plasma ACE level might be associated with an increased risk of coronary heart disease (CHD). To further assess this hypothesis, the activity of plasma ACE now has been measured in a large subsample of the ECTIM study, and the relation between plasma ACE level and MI has been analyzed.

Methods

Study Populations and Sampling of Patients and Control Subjects

The ECTIM study was designed to identify genetic factors involved in the development of MI. It consists of four inde-
Dependent studies in populations covered by World Health Organization/MONICA CHD registers: Belfast and its surroundings in Northern Ireland; Strasbourg and the department of Bas-Rhin in the northeast of France; Toulouse and the department of Haute-Garonne in the southwest of France; and Lille and its suburbs in the north of France.10 Men 25 to 64 years old were eligible for inclusion in the study; they had to reside in the region, and their parents and grandparents had to have been born in Europe (for the French centers) or in the historical entity of Ulster (Northern Ireland).

During the study period, all male patients surviving a definite MI (not necessarily a first MI), defined by MONICA criteria (category I), were eligible for the study. The patients were drawn from the MONICA registers, and further epidemiological data and blood samples were obtained at least 3 months, and at most 9 months, after the event.

Samples of population were obtained from the electoral rolls in France, and from the lists of general practitioners held by the Central Services Agency in Northern Ireland. Stratification by age was employed to match approximately the age distribution of the population samples with that of the patients. Among the eligible control subjects, 40% in Belfast, 54% in Strasbourg, 49% in Toulouse, and 47% in Lille refused to take part, did not respond, or could not be traced.

After informed consent was obtained, the control subjects were examined in clinics or, if necessary, at home by specially trained staff. A set of questionnaires was completed with details on personal history (including presence of CHD). The population samples included 9.4% of prevalent cases in Belfast and 4.3% in France. Subjects with CHD were excluded from the population samples to define the control groups. A questionnaire detailing all drugs taken by the subjects at the time of examination was administered by a technician who recorded all the brandnames. The generic name was later assigned centrally by a single person.

Plasma ACE Activity Measurement

Plasma was stored in liquid nitrogen for a mean period of 5 years. Plasma ACE activity was assessed blindly during a 2-month period on a series of batches, where samples from the different groups of patients and control subjects were mixed. As a consequence of transportation and storage problems, plasma ACE activity could not be measured in 24% of the patients and 22% of control subjects in the population samples; these percentages were 6% and 10% in Belfast, 11% and 7% in Lille, 14% and 21% in Strasbourg, and 69% and 40% in Toulouse, respectively.

Plasma ACE activity was measured on diluted plasma by quantifying the hydrolysis of hippuryl-histidyl-leucine after high-performance liquid chromatograph separation of the products as previously described.5 Measurements were performed at two different dilutions (1/125 and 1/250 final) for each plasma. Substrate consumption was kept ≤5%, and activity was determined in conditions of initial velocity. Results are expressed as units per liter, one unit corresponding to 1 micromole of substrate hydrolyzed per minute.

Genotyping of the ACE Gene

Insertion/Deletion Polymorphism

Genomic DNA was prepared from white blood cells by phenol extraction, and genotyping of the ACE I/D polymorphism was done as previously described.7

Statistical Analysis

Plasma ACE levels were compared between groups by ANOVA, using the GLM procedure of the SAS statistical software. Logistic regression (LOGIST procedure) comparing patients and control subjects was used to assess the independent effects of the ACE I/D polymorphism and plasma ACE level on MI risk after adjustment on covariates. The heterogeneity of association according to age was tested by introducing interaction terms in the ANOVAs. Adjustment on population was performed in all analyses. Since no significant heterogeneity across populations was detected, only global results are shown in the tables. Analysis of the distribution of plasma ACE in patients and control subjects was performed by commingling analysis,11 conditioned on marker genotype (see "Appendix").

Results

Plasma ACE activity and the ACE I/D polymorphism were assessed simultaneously in 1086 subjects (471 and 615 in the MI and population samples, respectively). The mean plasma ACE levels of the MI and population samples were 30.8 (SEM 0.5) U/L and 29.5 (0.4) U/L, respectively (P<.05) and were homogeneous across populations. The mean age of the two samples was similar: 54.0 (0.4) and 53.5 (0.3), respectively (nonsignificant difference). As expected, there was a strong association between plasma ACE and the ACE I/D polymorphism, the mean level of plasma ACE increasing with the number of D alleles (P<.0001 in patients and in control subjects). The percentage of variance of plasma ACE explained by the ACE I/D polymorphism was 18.4% in the patients and 20.0% in the population samples.

Plasma ACE and ACE Inhibitor Treatment

Since a number of subjects were treated with ACE inhibitors, the mean levels of plasma ACE were compared in the MI and population samples according to the ACE I/D genotypes and to ACE inhibitor treatment (Table 1). Sixty-four patients in the MI samples (13.6%) and 34 individuals in the population samples (5.5%) were treated with ACE inhibitors. In both groups, ACE levels were increased in the presence of treatment (P<.001). The interaction term involving treatment and genotype was highly significant, indicating a stronger effect of ACE inhibitors on plasma ACE level in subjects carrying the D allele than in II subjects. All further analyses were performed after exclusion of individuals treated with ACE inhibitors.

Plasma ACE and Personal History of Disease

In patients, the plasma level of ACE was unrelated to a personal history of recurrent MI, angina pectoris, stroke, hypertension, or diabetes; furthermore, no association with diuretic treatment was observed. In the population samples, a personal history of hypertension or diabetes was unrelated to plasma ACE activity, but those who had a history of CHD (MI or angina pectoris) (n=36) had higher plasma ACE levels than those without history: 32.7 (SEM 1.6) U/L and 29.3 (SEM 0.4) U/L, respectively (P<.05).

Case-Control Comparison of Plasma ACE Level and of the ACE I/D Polymorphism

The control groups include all members of the population samples having no personal history of CHD.

The population-adjusted odds ratio for MI of DD relative to ID+II individuals was 1.28 (0.95 to 1.74). Globally, no significant difference in plasma ACE levels between patients and control subjects was detected (Table 2). However, a strong interaction with age was present. As shown in Fig 1, in control subjects plasma ACE level did not vary with age (r=-.023, P=.6),
TABLE 1. Mean Plasma ACE Levels in Patients and Population Samples According to ACE Inhibitor Treatment and ACE I/D Genotypes

<table>
<thead>
<tr>
<th>ACE genotype</th>
<th>Receiving ACE Inhibitor</th>
<th>Population Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>DD</td>
<td>n=132</td>
<td>n=18</td>
</tr>
<tr>
<td></td>
<td>34.8 (0.9)*</td>
<td>50.3 (2.2)</td>
</tr>
<tr>
<td>ID</td>
<td>n=202</td>
<td>n=38</td>
</tr>
<tr>
<td></td>
<td>22.6 (1.2)</td>
<td>26.7 (3.4)</td>
</tr>
<tr>
<td>All</td>
<td>n=288</td>
<td>n=48</td>
</tr>
</tbody>
</table>

Statistical analysis†

<table>
<thead>
<tr>
<th></th>
<th>ACE inhibitor</th>
<th>ACE I/D</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>P&lt;.0001</td>
<td>P&lt;.0001</td>
<td>P&lt;.004</td>
</tr>
</tbody>
</table>

ACE indicates angiotensin-converting enzyme.

*Plasma ACE (U/L); means (SEM) adjusted on population. SEM shown in parentheses.
†ANOVA assessing the effect of ACE inhibitor and ACE genotype on plasma ACE level. No statistically significant heterogeneity across populations.

In patients, it significantly decreased with age \(r=-.225, \ P<.0001\). The test of homogeneity of the correlation coefficients was highly significant \(P<.001\). To take into account this interaction, the study population was divided according to the median of age (Table 2). In the group <55 years old, mean plasma ACE level was higher in patients than in control subjects within each ACE I/D genotype \(P<.01\), and the reverse was observed in those ≥55 years \(P<.07\). In individuals ≥60 years old, the difference was even more marked \(P<.01\).

The effects of plasma ACE level and of the ACE I/D polymorphism on the risk of MI were assessed by logistic regression analysis adjusted on population (Table 3). The two variables were tested separately because of the strong correlation between them. In the group <55 years old, plasma ACE level was a significant predictor of MI \(P<.008; P<.007\) after adjustment on other risk factors) but not ACE I/D. The opposite situation was observed in the group ≥55 years old: the ACE I/D polymorphism was related to the risk \(P<.07; P<.03\) after adjustment on other risk factors) but not plasma ACE. As a consequence of these two contrasting patterns, the ACE I/D polymorphism was only weakly associated with MI in the whole study population \(P<.07; P<.07\) after adjustment on other risk factors).

TABLE 2. Mean Plasma ACE Levels in Patients and Control Subjects, According to the ACE I/D Polymorphism and Stratified by the Median of Age

<table>
<thead>
<tr>
<th>ACE genotype</th>
<th>Age &lt;55</th>
<th></th>
<th>Age ≥55</th>
<th></th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients</td>
<td>Control Subjects</td>
<td>Patients</td>
<td>Control Subjects</td>
<td>Patients</td>
</tr>
<tr>
<td>DD</td>
<td>n=58</td>
<td>n=81</td>
<td>n=74</td>
<td>n=73</td>
<td>n=132</td>
</tr>
<tr>
<td></td>
<td>37.2 (1.1)*</td>
<td>35.3 (0.9)</td>
<td>33.2 (0.9)</td>
<td>33.1 (0.9)</td>
<td>35.0 (0.7)</td>
</tr>
<tr>
<td>ID</td>
<td>n=99</td>
<td>n=141</td>
<td>n=103</td>
<td>n=134</td>
<td>n=202</td>
</tr>
<tr>
<td></td>
<td>30.6 (0.9)</td>
<td>27.9 (0.7)</td>
<td>25.7 (0.8)</td>
<td>28.2 (0.7)</td>
<td>28.1 (0.6)</td>
</tr>
<tr>
<td>II</td>
<td>n=32</td>
<td>n=53</td>
<td>n=41</td>
<td>n=63</td>
<td>n=73</td>
</tr>
<tr>
<td></td>
<td>23.8 (1.5)</td>
<td>22.4 (1.2)</td>
<td>21.7 (1.2)</td>
<td>22.5 (1.0)</td>
<td>22.6 (1.0)</td>
</tr>
<tr>
<td>All</td>
<td>n=189</td>
<td>n=275</td>
<td>n=218</td>
<td>n=270</td>
<td>n=407</td>
</tr>
<tr>
<td></td>
<td>31.6 (0.8)</td>
<td>29.1 (0.6)</td>
<td>27.5 (0.6)</td>
<td>28.2 (0.5)</td>
<td>29.5 (0.5)</td>
</tr>
</tbody>
</table>

ACE indicates angiotensin-converting enzyme.

Subjects treated with ACE inhibitors and control subjects with coronary heart disease are not included.

*Plasma ACE (U/L); means (SEM) adjusted on age and population. SEM shown in parentheses.

Statistical analysis: ANOVA of plasma ACE adjusted on population and on the ACE I/D polymorphism comparing patients vs control subjects; all subjects: NS; age <55: \(P<.01\) (higher level in patients than in control subjects); and age ≥55: \(P<.07\) (lower level in patients than in control subjects).
Plasma ACE and MI in Low-Risk Subjects

Plasma ACE level also was investigated in the low-risk group, as defined in our previous report (subjects with a body mass index <26 kg/m², a plasma ApoB level <1.25 g/L, and not treated with hypolipidemic drugs). In Table 4 are shown the number and mean plasma ACE levels of patients and control subjects at low risk and according to genotypes. There was a higher percentage of DD genotypes in patients than in control subjects: 50% versus 28%, with a population and age-adjusted odds-ratio for MI of 3.0 (P<.0025). In the low-risk group, plasma ACE level was higher in patients than in control subjects: 31.6 versus 27.7 after adjustment on population and age (P<.01). However, a difference was observed only in the ID+II genotypes (28.5 in patients and 24.7 in control subjects, P<.01), whereas the mean ACE level was similar in patients and control subjects carrying the DD genotype.

Commingling Analysis of the Distribution of Plasma ACE

We have shown previously that the distribution of plasma ACE in families was compatible with the mendelian segregation of a major gene effect, possibly related to the presence of a functional polymorphism within or nearby the ACE gene (the postulated ACE Ss polymorphism). This polymorphism has not yet been characterized at the molecular level. The ACE I/D polymorphism is apparently only a marker in strong linkage disequilibrium with this functional polymorphism. It was therefore important to establish whether the effect of this putative major gene on plasma ACE could also be detected in the ECTIM study and if the differences in plasma ACE levels between patients and control subjects in the younger age group could be attributable to this genetic effect. For this purpose, a detailed analysis of the distribution of plasma ACE in patients and control subjects was performed by commingling analysis, incorporating information on the I/D polymorphism. Commingling analysis is a statistical technique used to assess whether the distribution of a quantitative variable is compatible with the presence of several underlying subdistributions and, if so, to estimate the number, mean, residual variance, and frequency of these subdistributions (see “Appendix”).

Fig 2 shows the distribution of plasma ACE levels in the whole study sample and within each ACE I/D

Table 3. Logistic Regression Analysis of Patient/Control Subject Status on Plasma ACE and ACE I/D Polymorphism, Respectively

<table>
<thead>
<tr>
<th>Logistic Regression Coefficient (SE)*</th>
<th>Patients</th>
<th>Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma ACE</td>
<td>.009 (.007) P&lt;.2</td>
<td></td>
</tr>
<tr>
<td>ACE I/D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age &lt;55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma ACE</td>
<td>.028 (.011) P&lt;.008</td>
<td></td>
</tr>
<tr>
<td>ACE I/D</td>
<td>.180 (.098) P&lt;.07</td>
<td></td>
</tr>
<tr>
<td>Age ≥55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma ACE</td>
<td>.009 (.011) P=.4</td>
<td></td>
</tr>
<tr>
<td>ACE I/D</td>
<td>.247 (.132) P&lt;.07</td>
<td></td>
</tr>
</tbody>
</table>

ACE indicates angiotensin-converting enzyme.
*Adjusted on population; †plasma ACE (U/L); ‡the ACE I/D polymorphism was entered in the models as an ordered (0,1,2) variable.

Table 4. Plasma ACE Level and ACE DD Genotype in Patients and Control Subjects at Low Risk

<table>
<thead>
<tr>
<th>ACE Genotype</th>
<th>Patients</th>
<th>Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n, %</td>
<td>Plasma ACE*</td>
</tr>
<tr>
<td>DD</td>
<td>27 (50)</td>
<td>34.3 (1.5)</td>
</tr>
<tr>
<td>ID+II</td>
<td>27 (50)</td>
<td>28.5 (1.5)</td>
</tr>
</tbody>
</table>

ACE indicates angiotensin-converting enzyme.
*Plasma ACE (U/L); means (SEM) adjusted on age and population.

Population and age-adjusted odds ratio for myocardial infarction associated with the DD genotype: 3.0 (P<.0025).
Case/control difference in mean plasma ACE level: DD genotype: NS; ID+II genotypes: P<.005.
ACE indicates angiotensin-converting enzyme.

*Commingling analysis of the three conditional distributions of plasma ACE: \( \mu_{SS} \), \( \mu_{sS} \), \( \mu_{SS} \) are the estimated means of the postulated genotypes ss, sS, and SS, respectively. \( \tau(S) \) is the probability of allele S, \( \tau(l|S) \) is the probability of allele I given s, and \( \sigma \) is the residual standard deviation.

Strategy used in the commingling analysis: The most general model (model 1) assumed that the genotype-specific means, the frequency of allele S, and the association between the Ss and I/D loci were different in patients and control subjects. Successive models then were tested with increasing numbers of parameters assumed identical in patients and control subjects. Comparison of successive models was made by the likelihood ratio test.

No departure from Hardy-Weinberg proportions at the postulated Ss locus was detected in either patients or control subjects. The association between alleles at the postulated Ss and observed I/D loci was similar in patients and control subjects (model 4 vs model 2). Marker I was preferentially associated with allele s, but its frequency conditional to allele S \( \tau(l|S) \) was significantly different from 0 \( (P<.01) \), indicating that the linkage disequilibrium between the susceptibility and marker loci was not complete.

Figs 3 and 4 show the distribution of plasma ACE levels in patients and control subjects <55 years and \( \geq 55 \) years, respectively. In the younger age group, the commingling analysis (Table 6, first column) gave good support for a mixture of three subdistributions with similar means and common SDs in patients and control subjects. The postulated allele S was more frequent in patients than in control subjects: 0.47 (SD 0.05) versus 0.36 (0.04), but the frequency of allele I conditional on S (probability for S to carry I) was also larger in patients than in control subjects 0.16 (0.04) versus 0.06 (0.03). The global difference for these two parameters between patients and control subjects was highly significant \( (P<.007) \). In the older subjects, the commingling analysis (Table 6, second column) did not reveal any difference in the distribution of plasma ACE between patients and control subjects. In control subjects, the frequency of the postulated allele S did not differ according to age, but in patients there was an apparent depletion of S in the older age group compared with the younger one (0.32 versus 0.47, \( P<.005 \)).

Fig 3. Bar graphs show distribution of plasma angiotensin-converting enzyme (ACE) according to ACE I/D genotypes in patients and control subjects <55 years. The plasma ACE distribution in DD, ID, and II genotypes is shown in black, gray, and white, respectively.
The data were compatible with a major effect of the putative ACE Ss polymorphism on plasma ACE levels and with a genetic explanation for the difference in mean plasma ACE level between patients and control subjects in the younger age group.

**Discussion**

This study confirms the strong association between the ACE I/D polymorphism and plasma ACE. In previous studies, the percentage of variance of plasma ACE explained by the ACE I/D polymorphism varied from 28% to 47%. In the present study, this percentage was about 20% in control subjects. The large differences in these percentages may be the consequence of random fluctuation or of different methods used to assess plasma ACE.

Plasma ACE activity in the absence of circulating inhibitors is proportional to the number of molecules of enzyme, as measured by radioimmunoassay. Increased plasma ACE levels have been observed repeatedly during chronic ACE inhibitor treatment and are probably a result of an induction of ACE synthesis in endothelial cells, most likely mediated at the transcriptional level. The regulatory factor responsible for the induction remains unknown. Induction was observed in this study probably because blood was taken at distance from the last drug intake and also because of the lack of stability of sulfhydryl-containing inhibitors in plasma samples. Also, the high dilution of the sample in the assay might have favored the dissociation of the ACE-inhibitor complex if residual inhibitor was present in the plasma. A larger induction was observed in this study in subjects heterozygote or homozygote for the D allele. The influence of the polymorphism on induction was codominant in patients, whereas in the population samples the effect was weaker in DD homozygotes than in heterozygotes. This is probably a chance finding resulting from the small number of treated subjects, homozygotes for allele D, in the population samples. The genotype-dependent induction of ACE by ACE inhibitors suggests that the ACE functional polymorphism not only alters the basal level of transcription but also modulates the ACE gene response to regulatory factors. This may be similar to the genotype-dependent increase in plasma ACE levels previously reported in children. Induction of ACE gene expression also has been observed in hypertrophied hearts and in vascular smooth muscle cells after endothelial injury in the rat. If this phenomenon occurs in humans, it would be of interest to determine whether it is also modulated by the ACE gene polymorphism. In our study, the prescription of ACE inhibitors was not controlled; the genotype-dependent induction of ACE synthesis by ACE inhibitors therefore should be considered a hypothesis, which will have to be tested in controlled studies where plasma ACE level is assessed before and during treatment.

We previously reported that the ACE DD genotype was positively associated with MI in the ECTIM study with a population-adjusted odds ratio of 1.34 when compared with ID+II individuals. The smaller odds ratio estimated in the present analysis (1.28) is due to the noninclusion of individuals for whom measurement of plasma ACE was not available or who were treated with ACE inhibitors. Overall, 24% of patients and 22% of control subjects were excluded for these reasons, most of them from France. This tended to increase the proportion of participants from Belfast in the sample and to lower the odds ratio for MI associated with the DD genotype, since no association was found between the polymorphism and MI in the whole sample in Belfast. In the low-risk group, however, the odds ratio for MI of DD versus ID+II individuals was quite similar to that found in the previous analysis of the ECTIM data (3.0, P<.0025).

From our previous family studies, we suspected that the ACE I/D polymorphism was only a marker for a putative functional variant (denoted Ss) controlling
plasma ACE level. It was thus of interest to determine whether plasma ACE level provided additional information to the I/D polymorphism regarding the contribution of the ACE gene to the risk of MI in the ECTIM study. Plasma ACE level was very homogeneous across the four populations, was unrelated with age in control subjects, but decreased with age in patients. As a consequence, in the older subjects, patients tended to have a lower mean level of plasma ACE than control subjects whereas in younger subjects, patients had consistently higher mean levels of plasma ACE than control subjects within the three ACE I/D genotypes. This interaction could reflect the fact that plasma ACE is a risk factor only in the younger age group but also is compatible with the underrepresentation of patients with high plasma ACE levels in older MI subjects (see detailed discussion below).

Given the important differences in plasma ACE levels between patients and control subjects younger than 55 years and the lack of difference in older individuals, a series of commingling analyses of plasma ACE were performed to see whether this observation could receive a genetic interpretation. The results of the analyses in the whole sample demonstrated the presence of three subdistributions of plasma ACE, which probably correspond to the three putative ACE genotypes ss, Ss, and SS. The modes of these subdistributions were found at the same plasma ACE levels in patients and control subjects and were compatible with the genotypic means inferred from the segregation analysis of plasma ACE in families. The percentage of variance of plasma ACE explained by the putative Ss polymorphism was approximately 60% in both patients and control subjects; this value is very close to that inferred from family studies and should be compared with the 20% of variance of plasma ACE level explained by the ACE I/D marker polymorphism. The main conclusions of the commingling analyses were:

(1) Before age 55, the postulated allele S was more frequent in patients than in control subjects: 0.47 and 0.36, respectively, but the genotype-specific means were not different between patients and control subjects. This result suggests that the case-control difference of plasma ACE level observed in this age group was explained largely by the different frequencies of the postulated allele S in patients and control subjects. The predicted frequencies of the postulated genotypes SS, Ss, and ss, assuming random pairing of alleles, were, respectively, 0.22, 0.50, and 0.28 in patients and 0.13, 0.46, and 0.41 in control subjects. If codominance is assumed, the derived odds-ratios for MI of SS and Ss with respect to ss were 2.5 and 1.6, respectively.

(2) The frequency of the postulated allele S in patients dropped from 0.47 before age 55 to 0.32 after (P < .005). As already suggested, high levels of plasma ACE could be a risk factor for MI only in the younger individuals. However, it is important to consider that this age-related difference in allele frequency could also be the consequence of selection by death, if those developing an MI and carrying S were more prone to die from their disease. This possibility is in accordance with a couple of observations made in the ECTIM study. First, we have shown that the ACE DD genotype was more strongly related to MI in subjects at low risk of atherosclerosis than in high-risk subjects. A possible explanation for this finding is selection by death of high-risk DD individuals. Second, parental history of fatal MI was associated with the ACE D allele in the offspring, and this association was stronger than what would have been predicted from the difference between patients and control subjects. Again this discrepancy could be the consequence of a high lethality in DD patients with MI, which could affect the case-control comparison. In the ECTIM study, patients were included 3 to 9 months after occurrence of MI. Several sources of evidence suggest that approximately 30% of the patients who develop an acute coronary event die before hospitalization and that during the first 3 months after hospitalization, about 8% of the hospitalized patients die from a complication of their MI. Such selection could strongly affect the frequency of a risk factor for death in MI survivors. The results of a recently completed study of the ACE I/D polymorphism in patients who died of an MI in Belfast have confirmed that deceased patients have a higher frequency of the ACE D allele than control subjects.

Although the difference between patients and control subjects of plasma ACE in the younger age group was compatible with the presence of an underlying major gene effect, we cannot exclude the possibility that plasma ACE activity might also be altered in the post-MI phase, even 3 to 9 months after disease onset. This is a usual shortcoming of phenotype measurement in retrospective studies. However, several arguments suggest that the higher mean ACE level in cases is not secondary to MI: (a) The modes of the subdistributions of plasma ACE in the three ACE I/D genotypes were similar in patients and control subjects. In subjects ≤ 55 years old (Fig 3), the higher mean ACE level in patients than in control subjects was apparently the consequence of an increased frequency of the postulated Ss and SS genotypes. (b) The increased ACE level in MI patients was observed only in the younger age group; if it was a post-MI change, it should probably be observed in the older subjects as well. (c) Results obtained in rats suggest that, in the presence of experimental MI, plasma ACE level remains normal and increases only when congestive heart failure occurs (Huang et al, unpublished results). In the ECTIM study, few patients had congestive heart failure and most of them were not included in the analysis because they were receiving ACE inhibitors. Among those receiving diuretics (n = 49), no increase in plasma ACE activity was noted. (d) Finally, no alterations in plasma ACE levels have been observed in humans following the occurrence of MI.

(3) The postulated allele S was found mainly on the marker allele D, but there was no complete linkage disequilibrium between the two loci, since allele I was also unambiguously present on allele S. This was shown by the greater than 0 probability r(I|S) in the commingling analysis. From our former family study, we concluded in favor of a complete linkage disequilibrium [r(I|S) = 0] between the postulated Ss and the observed I/D loci. This was probably the consequence of a lack of power of this family study.

The absence of association between ACE I/D and MI in the younger age group despite the higher frequency of the postulated allele S in patients than in control subjects was explained by the lower probability of allele
D given the postulated allele S (0.84 versus 0.94) in the MI patients than in control subjects (Table 6). This puzzling observation could be the consequence of random fluctuation. However, it could also have a biological support, if the ACE I and/or ACE D alleles carry other functional variants of less importance than S, but which nevertheless significantly affect the level of ACE and the risk of MI. This crucial point probably will be resolved when ACE S and other possible functional variants are identified.

In conclusion, our results suggest that plasma ACE measurement provides information that complements that given by the ACE I/D polymorphism for assessing the contribution of the ACE locus to MI. The commingling analysis conditional on the ACE I/D polymorphism enabled us to estimate the frequency of the postulated S allele and the amplitude of its effect on MI risk. It will be interesting to compare these estimates with the observed values when the Ss polymorphism is identified at the molecular level. The results extend those reported previously by showing that plasma ACE level is associated with MI and that this association is largely of genetic origin.

Appendix

Commingling Analysis of the Distribution of a Phenotype (Plasma ACE) Conditioned on a Marker Genotype (ACE I/D)

Commingling analysis tests the compatibility of an observed distribution with a mixture of several normal distributions with different means and identical variance. If the mixture is caused by an underlying polymorphic gene locus, the respective frequencies of the subdistributions are the genotype probabilities. If the population is in Hardy-Weinberg equilibrium, these probabilities are determined by the Hardy-Weinberg proportions. The parameters of the model are then the genotype-specific means, the within-genotype common SD, and the allele frequencies. An additional parameter can be included to relax the Hardy-Weinberg assumption. These parameters are estimated by likelihood maximization. If the analysis is conditioned on the genotype of a marker tightly linked to the susceptibility locus, the resolution of commingling analysis is increased. In this case, the likelihood function is written as

$$L(x|m) = \sum_k P(x|m|k)P(g) = \sum_k P(x|g)P(m|g)P(g)$$

where $x$ is the trait, $m$ is the marker genotype, and $g$ is the susceptibility genotype underlying the trait. $P(g)$ is the prior probability of the genotypes. If we denote S and D the two alleles at the susceptibility locus and I and D the two alleles at the marker locus, $P(g)$ and $P(m|g)$ are parameterized in terms of $\pi(S)$, the frequency of S, and $\pi(S)$ and $\pi(D)$, the frequencies of I conditional to allele S and D, respectively. These conditional probabilities are functions of the linkage disequilibrium between the two loci. Under linkage equilibrium $\pi(I|S) = \pi(I|S)$, $P(g|x)$ is the penetrance function conditional on the genotype underlying the trait. It is assumed to be distributed normally with genotype-specific means $\mu(SS)$, $\mu(SD)$, and $\mu(DD)$, and common SD $\sigma$. In the more general model, parameters were allowed to differ between patients and control subjects. Specific hypotheses were tested by means of the likelihood ratio test.

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