Prospective evaluation of the effect of an angiotensin I converting enzyme gene polymorphism on the long term risk of major adverse cardiac events after percutaneous coronary intervention

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Objective: To evaluate prospectively the influence of an angiotensin I converting enzyme (ACE) gene polymorphism on long term clinical outcome of patients with established coronary artery disease treated by percutaneous coronary intervention.

Design and setting: Prospective observational study in a university hospital.

Patients: Consecutive series of 1010 patients with symptomatic coronary artery disease who underwent successful coronary stent placement from November 1996 to April 1998.

Main outcome measures: Long term clinical outcome was obtained and the rates of major adverse cardiac events (death, non-fatal acute myocardial infarction, unstable angina, and revascularisation) were compared according to the insertion/deletion (I/D) polymorphism of the ACE gene.

Results: Of the 1010 patients 29% had the DD genotype, 51% had the ID genotype, and 20% had the II genotype. All baseline clinical angiographic and procedural characteristics were identical in the three groups of patients. Event-free survival during the follow up period (median two years) was identical in patients with the II genotype compared to those with one or two D alleles. The predictors of long term survival were age, diabetes, ejection fraction, and extension of coronary artery disease. ACE genotype had no influence on the long term survival. Additional analyses assuming dominant and recessive effects of the D allele also failed to find any association; nor did the examination of low risk subgroups.

Conclusions: The ACE I/D polymorphism does not influence the long term prognosis of patients with coronary disease treated by percutaneous coronary intervention, and screening patients for this gene polymorphism is not useful for secondary prevention strategies.

METHODS
Primary end point
This prospective observational study was designed to examine the relation between ACE I/D gene polymorphism and the long term risk of MACE (death, acute myocardial infarction, unstable angina, and coronary revascularisation) in patients with symptomatic coronary artery disease treated by PCI. The primary outcome was a composite of MACE. Secondary endpoints were individual and combined outcomes of death, acute coronary syndromes, or any revascularisation.

Patients
From November 1996 to April 1998, 1039 consecutive white patients with symptomatic coronary artery disease who had successful PCI with stent implantation were prospectively studied. All patients gave written informed consent for the study according to our institutional ethics committee recommendations. Among the study population eight patients (0.8%) were definitively lost to clinical follow up and genotype was not available or not identified in 21 patients (2%). The remaining 1010 patients constituted the study cohort.

Abbreviations: ACE, angiotensin I converting enzyme; MACE, major adverse cardiac events; MONICA, monitoring trends and determinants in cardiovascular disease; PCI, percutaneous coronary intervention; PCR, polymerase chain reaction
Statistical analysis
Baseline characteristics of the study population are presented as counts and percentages for categorical variables and as mean (SD) for continuous variables. Differences in percentages were evaluated by the \( \chi^2 \) test and means by analysis of variance. Validity conditions were checked for each comparison. No striking deviation from the Hardy-Weinberg equilibrium was observed in the distribution of ACE I/D gene polymorphism (\( \chi^2 = 1.19, p > 0.50 \)). Univariate analysis with the Kaplan-Meier product limit method and the log rank or Breslow tests was used to compare the genotype groups.

For identification of prognostic factors related to survival time and the relation between ACE I/D polymorphism and outcome, Cox's proportional hazards models were used after adjustment for variables proved to be significant with univariate statistical analysis (\( p < 0.20 \)). The relative risks are given with a 95% confidence interval. Significance was covered by an \( \alpha \) error of 0.05. All calculations were performed with SPSS Professional Statistics 10.0.7 (SPSS Inc, Chicago, Illinois, USA).

RESULTS
Baseline characteristics of the study population
There were no differences in baseline clinical and angiographic characteristics between the three groups of genotypes (table 1). The study population was mainly treated and dilated for acute coronary syndromes (acute myocardial infarction or unstable angina) with the same proportion of patients in each group (74%, 79%, and 80% for DD, ID, and II genotypes, respectively). Diabetic patients were similarly represented in the three groups of patients. At hospital discharge, 35–44% of patients were receiving ACE inhibitors with no significant difference between the three groups of patients. Table 1 shows the distribution of several other risk factors in the three groups of genotypes. The distribution reflects the expected prevalence of recognised risk factors in patients with established coronary artery disease. No comparison between the three groups of genotypes was significant.

Frequencies of alleles and genotypes
The frequencies of the I and D alleles were 45% and 55%, respectively. The frequencies of the DD (29%), ID (51%), and II (20%) genotypes were virtually identical to those predicted by the Hardy-Weinberg equilibrium. None of the recognised risk factors shown in table 1 differed in distribution or in mean value from those of the ACE genotype.

Clinical follow up of patients
During follow up the primary clinical end point, a composite of MACE, was reached in 35%, 37%, and 34% of patients with the DD, ID, and II genotypes, respectively.
with no significant difference. During a mean follow up period of 2 (1) years (maximum 4 years) a mortality of 5%, 8%, and 6% was observed in patients with the DD, ID, and II genotypes, respectively, with no significant difference between groups. Table 2 details other MACE, with no differences between genotypes. Kaplan-Meier analysis showed that patients in the three groups of genotypes had the same probability of death, revascularisation rates, or composite of MACE during the entire follow up period (fig 1). Ischaemia driven target and non-target vessel revascularisations were not influenced by the ACE genotype (results not shown). Additional analyses assuming a dominant or a recessive effect of the D allele did not change the results. Analyses were also performed with adjustment for PCI indication and ACE inhibitor treatment and in a low risk subgroup defined by a body mass index below 25 kg/m² and with no dyslipidaemia. No association between ACE genotype and clinical outcome was observed (data not shown).

Multivariate analysis
Multivariate analyses of the relation between clinical data, angiographic findings, genetic markers, conventional risk factors, and the risk of MACE showed that four variables were significant: the presence of diabetes, prior percutaneous transluminal coronary angioplasty, low ejection fraction, and multivessel disease. ACE I/D genotype was not associated with deleterious prognosis either in dominant or in recessive assumptions. Table 3 shows the hazard ratios and the 95% confidence intervals for the independent prognostic variables.

Figure 1 Kaplan-Meier estimates of the composite outcome of major adverse cardiac events in relation to angiotensin converting enzyme insertion/deletion genotypes with no significant difference observed.

Table 2 Incidence of the primary outcome and other outcomes according to ACE genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Composite of MACE (%)</th>
<th>Death (%)</th>
<th>Revascularisation (%)</th>
<th>PTCA</th>
<th>CABG</th>
<th>Unstable angina (%)</th>
<th>AMI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD (n=297)</td>
<td>35</td>
<td>5</td>
<td>15</td>
<td>15</td>
<td>4</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>ID (n=517)</td>
<td>37</td>
<td>8</td>
<td>18</td>
<td>18</td>
<td>3</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>II (n=196)</td>
<td>34</td>
<td>6</td>
<td>16</td>
<td>16</td>
<td>3</td>
<td>14</td>
<td>1</td>
</tr>
</tbody>
</table>

MACE, major adverse cardiac events.

Table 3 Relative risk (RR) for composite of MACE: results of multivariate Cox regression model

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Composite of MACE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>1.35 (1.00 to 1.84)</td>
</tr>
<tr>
<td>LVEF &lt;40%</td>
<td>1.36 (1.06 to 1.77)</td>
</tr>
<tr>
<td>Multivessel disease</td>
<td>1.51 (1.10 to 1.77)</td>
</tr>
<tr>
<td>Prior PTCA</td>
<td>1.52 (1.10 to 2.09)</td>
</tr>
<tr>
<td>DD v ID v II</td>
<td>1.02 (0.73 to 1.42)</td>
</tr>
<tr>
<td>DD v ID v II</td>
<td>1.02 (0.76 to 1.38)</td>
</tr>
<tr>
<td>DD v ID v II (dominant)</td>
<td>1.02 (0.77 to 1.36)</td>
</tr>
<tr>
<td>DD v ID v II (recessive)</td>
<td>1.00 (0.79 to 1.28)</td>
</tr>
</tbody>
</table>

*p Value

DISCUSSION
As expected from the results of previous studies concerning patients with documented coronary artery disease, we found that several baseline variables including the presence of diabetes, the extent of coronary artery disease, and left ventricular ejection fraction were important and independent predictors of the risk of MACE after PCI. However, in this large prospective observational study we failed to observe any influence of the ACE I/D genotype on the long term risk of MACE after PCI.

Although there is evidence of a genetic predisposition for coronary artery disease, the impact of single genetic polymorphisms on the pathogenesis and natural history of ischaemic heart disease remains uncertain. The association of ACE I/D gene polymorphism with the risk of ischaemic heart disease in a case control study was provocative and generated great interest, although larger prospective studies in different study populations found no interaction. Because there is a strong correlation between ACE I/D genotypes and plasma ACE activity, and given the enormous number of studies that support the concept that blocking ACE by ACE inhibitors is beneficial for patients with congestive heart failure, acute myocardial infarction, and coronary artery disease, the concept that the DD genotype confers an increased risk of myocardial ischaemia is plausible. Indeed several biological actions of ACE may be involved in the pathogenesis of coronary artery disease: both the transformation of angiotensin I to angiotensin II, a potent vasoconstrictor, and the inactivation of bradykinin potentially result in decreased myocardial perfusion; angiotensin II induced stimulation of plasminogen activator 1 may favour the formation of occlusive thrombi; and angiotensin mediated promotion of smooth muscle cells growth may be involved in the progression of coronary artery disease. However, our findings in a large prospective study do not support the postulated role of the ACE genotype as a marker for long term risk of MACE in patients with established coronary artery disease who need PCI. Up to now, the influence of the ACE I/D gene polymorphism on the long term risk of MACE has not been studied. Some studies have reported an interaction of this polymorphism with in-stent restenosis, but these results have not been confirmed by larger studies.

In the present study no difference was observed in the various outcomes, including revascularisation rates, between the three genotypes. A recent report on a small series of patients suggested that ACE inhibitors given to patients with DD genotype may be associated with an unexpected deleterious effect on angiographic restenosis and with a trend for an increased risk of MACE. Our study, in a large patient cohort, does not support the hypothesis that an interaction between ACE inhibitors and different
ACE I/D gene polymorphism is not a useful marker for assessing risk of MACE associated with the DD genotype. Thus, our findings suggest that, at least in our regional population, the concept that other important factors can overcome the overall impact of a particular single gene polymorphism, such as the ACE I/D polymorphism. However, screening for new susceptibility genetic factors and long term risk of MACE in ischaemic heart disease may allow us to identify in the future the specific individual genetic risk of our patients. Apart from biological explanations, statistical considerations are also important in assessing the power of a study to detect or to exclude a potential effect. From this perspective our study had 90% power (α = 0.05) to detect a 1.3-fold increase in risk of MACE associated with the DD genotype. Thus, our findings suggest that, at least in our regional population, the ACE I/D gene polymorphism is not a useful marker for assessing the long term risk of patients with ischaemic heart disease.

On the basis of the successful reduction of morbidity and mortality after myocardial infarction or in high risk patients by treatment with ACE inhibitors,26 27 28 29 we believe that the renin-angiotensin system probably has an important role in ischaemic heart disease. However, the mechanism has so far remained elusive. Our results do not exclude a pathogenic role for mutations of the ACE gene in ischaemic heart disease. However, screening for new susceptibility genetic factors and long term risk of MACE in ischaemic heart disease may allow us to identify in the future the specific individual genetic risk of our patients. Apart from biological explanations, statistical considerations are also important in assessing the power of a study to detect or to exclude a potential effect. From this perspective our study had 90% power (α = 0.05) to detect a 1.3-fold increase in risk of MACE associated with the DD genotype. Thus, our findings suggest that, at least in our regional population, the ACE I/D gene polymorphism is not a useful marker for assessing the long term risk of patients with ischaemic heart disease.

Coronary artery disease is a multifactorial disease. Many environmental and, potentially, genetic factors contribute to the development of ischaemic heart disease. Our study of a large cohort of patients with clearly established ischaemic heart disease examined a study population coming from a single high volume centre characterised by regional environmental influences. Our study population may differ from those of multicentre studies with heterogeneous populations (for example, the World Health Organization Heart Health Study MONICA (monitoring trends and determinants in cardiovascular disease) survey). The negative results of our study underline the concept that other important factors can overcome the overall impact of a particular single gene polymorphism, such as the ACE I/D polymorphism. However, screening for new susceptibility genetic factors and long term risk of MACE in ischaemic heart disease may allow us to identify in the future the specific individual genetic risk of our patients. Apart from biological explanations, statistical considerations are also important in assessing the power of a study to detect or to exclude a potential effect. From this perspective our study had 90% power (α = 0.05) to detect a 1.3-fold increase in risk of MACE associated with the DD genotype. Thus, our findings suggest that, at least in our regional population, the ACE I/D gene polymorphism is not a useful marker for assessing the long term risk of patients with ischaemic heart disease.
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9. Enter/amend your contact information, and update your expertise data.