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

M Hamon, S Fradin, A Denizet, E Filippi-Codaccioni, G Grollier, R Morello

**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 end points 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.
The hospital records of patients included in the study were systematically reviewed by a physician who recorded demographic and clinical data including age, sex, medical history, cardiovascular risk factors, complications during hospitalisation, ECG findings, angiographic characteristics, dilatation procedures, and medications.

Genetic analysis
Blood samples were obtained at the end of the procedure after stent deployment. Genomic DNA from each patient was prepared from peripheral leucocytes by the salt precipitation method. The D and I alleles were identified on the basis of polymerase chain reaction (PCR) amplification of the respective fragments from intron 16 of the ACE gene as previously described. Amplification of the D allele results in a 190 bp fragment and amplification of the I allele results in a 490 bp fragment. Each sample that had the DD genotype underwent PCR amplification with a primer pair that recognises an insertion specific sequence. If the DNA was mistyped, a PCR product of 335 bp is present. Otherwise, no PCR product appears.

Follow up
Follow up was obtained by mailed questionnaires and scripted telephone interviews conducted by a physician. Events were verified by contacting the patient’s primary physician and reviewing medical records and death certificates. The primary clinical end point considered was a composite of hard cardiac events defined as non-fatal Q wave myocardial infarction or unstable angina with the same proportion of patients in each group (71%, 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.

**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 remains uncertain. The association of 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
genotypes has any significant effect on long term clinical outcome.

Our study focused on the long term clinical outcome of a large consecutive series of patients with angiographically documented coronary artery disease. In this well selected high risk study population, with nearly 35% of patients reaching the primary study composite outcome and with a global mortality rate of 6.3% over the clinical follow up period, no influence of the ACE I/D gene polymorphism was found. Because it has been suggested that the II genotype may delay the onset of acute coronary syndromes, we compared the rate of MACE during the study period. However, as shown by the Kaplan-Meier estimates, the three groups of genotypes had the same probability of death, revascularisation rates, or composite of MACE during the entire follow up period.

Our study does not rule out the possibility that certain mutant alleles of the ACE gene may be associated with a predisposition to MACE after PCI; our results simply indicate that the ACE I/D polymorphism does not serve as a useful marker of a putative disease causing mutation on the ACE gene. Recently, a number of small case–control studies found associations between the prevalence of the ACE genotype and various aspects of coronary artery disease with contradictory results. It is important to recognise that these case control studies of linkage disequilibrium (association) are highly sensitive to the selection of a genetically appropriate control sample. In the prospective evaluation of a cohort of patients the sample size is also critical. It is interesting to note that our earliest preliminary results were flawed by a chance of major significance after PCI; our results simply indicate that the II genotype has any significant effect on long term clinical outcome. 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, but indicate for the first time that the ACE I/D polymorphism is not a useful marker for assessing the long term risk of MACE after PCI or for secondary prevention strategies.

Authors’ affiliations

M Hamon, A Denizet, E’Filippi-Codaccioni, G Grollier, Department of Cardiology, Centre Hospitalier Universitaire de Caen, Caen, France
S Fradin, Department of Biochemistry, Centre Hospitalier Universitaire de Caen
F Corello, Department of Statistics, Centre Hospitalier Universitaire de Caen

*Also UPRES EA 3212, Normandy, France

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