*Br Heart J* 1995;74:584–591

# Angiotensin converting enzyme gene deletion allele is independently and strongly associated with coronary atherosclerosis and myocardial infarction

Eloisa Arbustini, Maurizia Grasso, Roberta Fasani, Catherine Klersy, Marta Diegoli, Emanuele Porcu, Nadia Banchieri, Paolo Fortina, Cesare Danesino, Giuseppe Specchia

**Abstract**

***Objective*—To investigate the association of the three angiotensin converting enzyme (ACE) genotypes, *DD*, *ID*, and *II*, with the occurrence or absence of coronary atherosclerosis and with myocardial infarction and hypertension.**

***Design*—Cohort analysis study.**

***Setting*—North-Italy reference centre.**

***Subjects*—388 white Italian patients (281 males; mean age 60·7 (SD 12·5) years) with proven coronary atherosclerosis (n = 255) or with angiographically normal coronary arteries (n = 133). A further group of 290 healthy blood donors was tested for allele frequency comparison.**

***Interventions*—ACE*/ID* polymorphism was analysed with polymerase chain reaction on DNA from white blood cells.**

***Main outcome measures*—Coronary atherosclerosis, myocardial infarction, hypertension.**

***Results*—The *D* and *I* allele frequencies were respectively 0·63 and 0·37 in the overall healthy blood donor group and 0·66 and 0·34 in the overall study group. In the latter, univariate analysis showed (1) that coronary atherosclerosis (255 patients) was associated with the deletion allele, with an odds ratio (OR) of 5·78 for *DD/II*, P < 0·001, and 2·39 for *ID/II*, P = 0·006; and (2) that myocardial infarction (154 patients) was associated with the *DD* genotype (OR *DD/II* = 2·56, P = 0·007), but not with the *ID* genotype (OR *DD/II* = 1·96, P = 0·056). Finally, hypertension proved to be unrelated with the ACE genotype. The distribution between the three genotypes of known risk factors for coronary artery disease was similar. Logistic regression modelling, performed to test the association of the selected risk factors simultaneously with coronary atherosclerosis and myocardial infarction, showed that the deletion allele (whether *DD* or *ID*) was the strongest risk factor for atherosclerosis, and that the *D* allele was significantly associated with the risk of infarction (although to a lesser extent than with coronary atherosclerosis).**

***Conclusion*—ACE deletion polymorphism is strongly and independently associated with coronary atherosclerosis and, to a lesser extent, with myocardial infarction. As such, the results are analogous to what has already been reported in French white, Japanese, and Welsh coronary patients.**

(*Br Heart J* 1995;74:584–591)

Keywords: ACE gene, insertion/deletion polymorphism, coronary atherosclerosis, myocardial infarction

**Pathological Anatomy, University of Pavia, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico S. Matteo, Pavia, Italy**
E Arbustini
M Grasso
R Fasani
M Diegoli
E Porcu
N Banchieri

**Biometric Unit—Scientific Division, University of Pavia, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico S. Matteo, Pavia, Italy**
C Klersy

**Cardiology Department, University of Pavia, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico S. Matteo, Pavia, Italy**
G Specchia

**The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA**
P Fortina

**General Biology and Medical Genetics, University of Pavia, Italy**
C Danesino

Correspondence to:
Dr Eloisa Arbustini, Istituto di Anatomia Patologica, Via Forlanini 16, 27100 Pavia, Italy.

Accepted for publication 14 July 1995

The deletion (*D*) or the insertion (*I*) of a 250 base pair (bp) DNA fragment in the angiotensin I converting enzyme (ACE) gene accounts for the three main ACE genotypes, *II*, *ID*, and *DD*.[1] About 50% of total phenotypic variance in serum ACE corresponds to this insertion/deletion polymorphism: the concentration of serum ACE is lowest in homozygotes with the longer allele (*II*) and highest in homozygotes with the shorter allele (*DD*); *ID* heterozygotes show intermediate levels.[2–4] Deletion polymorphism has been found to be a potent risk factor for myocardial infarction, both in white French patients otherwise defined as low risk on account of the absence of cardiovascular risk factors,[5] and in a recently reported US population.[6] The same polymorphism has been associated with a family history of fatal myocardial infarction in a part of the ECTIM population.[7] More recently, it has been reported that ACE gene deletion is strongly associated with coronary artery disease in French white patients with non-insulin-dependent diabetes mellitus,[8] in Japanese coronary patients,[9] and in a Welsh community.[10] Furthermore, the same polymorphism proved to be associated with the so called "ischaemic cardiomyopathy",[11] in which severe and diffuse coronary atherosclerosis and myocardial infarction coexist.[12] Conversely, a prospective case-control study of US male physicians failed to show an association between the ACE *D* allele and a significantly increased risk of clinically overt coronary artery disease.[13] Thus, whether or not the ACE locus may represent a genetic determinant for coronary artery disease is still a matter of debate. The question, as rightly raised by Lindpaintner *et al*,[13] as to whether an intronic *ID* polymorphism is in itself a disease causing mutation, does not represent a limit to the value of *ID* polymorphism as a genetic marker of a disease associated mutation (this marker

could exist in linkage disequilibrium with a putative pathogenetic mutation elsewhere in the ACE gene).

On the basis of these considerations the topic deserves further investigation. We accordingly examined ACE gene polymorphism in Italian white patients with and without angiographically proven coronary atherosclerosis and with and without myocardial infarction. A further group of healthy blood donors was tested for ACE gene *ID* allele frequency.

## Methods

### STUDY POPULATION

The study group consisted of 388 white patients (M281, F107) aged 60·7 (SD 12·52) years (range 15–92) with one of the following: coronary artery disease, valvar disease, idiopathic cardiomyopathy, congenital heart disease, atypical chest pain, cardiac amyloidosis, cardiac mixoma, tymoma, and muscle dystrophy. Standardised clinical and instrumental diagnostic criteria were adopted for the diagnosis of all these diseases.[14] The risk factor status for coronary artery disease was available for all patients.

The patients were subgrouped on the basis of the presence or absence of coronary artery pathology, as variously defined by: (1) coronary angiography; (2) occurrence of either single or multiple myocardial infarctions; (3) pathological study of necropsy or transplanted hearts. Given the aim of the investigation, we had to compare patients with angiographically proven normal coronary arteries (that is, absence of angiographically evident luminal narrowings, true control group) with patients with diseased coronary arteries (true test group). Since we needed to satisfy the requirements that enrolment be *in vivo* and that normal coronary artery patients be age matchable with those affected by coronary artery disease, we included non-ischaemic cardiac diseases which, for diagnostic and therapeutic purposes, required a coronary angiography.

On the basis of the occurrence or the absence of coronary atherosclerosis, we grouped all cases as: (1) patients with atherosclerotic coronary arteries; (2) patients with normal coronary arteries. The occurrence or absence of coronary atherosclerosis was assessed on the basis of the following.

(1) *Coronary artery status*, as documented in 286 patients by coronary angiography in the current (n = 163) or in previous (n = 115) admissions to our own or other hospitals, or by pathology on necropsy (n = 6) or explanted heart (n = 2) studies. We used standard angiography diagnostic criteria to define the occurrence of coronary artery disease.[15,16] The occurrence of both critical and subcritical luminal narrowings was considered as the expression of coronary atherosclerosis, and was the criterion for the inclusion of the case in the coronary artery disease group.

(2) *Acute* (n = 20) *or previous myocardial infarction* (n = 82), either single (n = 71) or multiple (n = 31, 11 of whom had ischaemic cardiomyopathy). In this group there were no patients with endocarditis, prosthetic valves, oral contraceptive use, viral illnesses, or other conditions which are known, albeit rarely, to cause myocardial infarction with non-atherosclerotic coronary arteries.[17–21]

The interval between coronary angiographies performed on other than the present hospital admission varied from months to years. *Angiographically positive cases* were included irrespective of this interval, since they were undoubtedly positive (this group included, for example, patients who had undergone previous surgical revascularisation or coronary angioplasty, or patients with valvar heart disease and associated coronary artery disease). For *negative cases* (patients with angiographically normal coronary arteries), we only included those patients who underwent coronary angiography on the current hospital admission or who had recently (< one year) undergone angiography and had been followed up in our cardiology department. This group consisted of idiopathic dilated cardiomyopathies, valvar heart diseases without coronary atherosclerosis, atypical chest pain with normal coronary arteries (X syndrome), and a minor series of rarer disorders, such as cardiac myxoma, amyloidosis, and so on.

We further tested 290 randomly selected blood donors (210 were males) aged 35 (SD 13) years, with normal physical examination, normal electrocardiogram, normal blood pressure, normal major lipid and coagulation variables, and normal viral tests (hepatitis B and C, HIV). This part of the study was only aimed at assessing whether the incidence of *D* and *I* allele frequency in our study population was similar to that in the general healthy population.

### RISK FACTORS FOR ATHEROSCLEROSIS

#### *Hyperlipidaemia*

Hyperlipidaemia was defined as plasma cholesterol or triglyceride levels or both above the 95th centile value for age and sex, according to the cholesterol consensus conference criteria.[22] Previous and present cholesterol and triglyceride levels were collected from each patient. Cholesterol and triglycerides were measured with enzymatic colorimetric methods on fasting blood samples.[23] Given that several patients had modified their lifestyle (dietary intake of cholesterol and saturated fats, or pharmacological treatment for hyperlipidaemia), the hyperlipidaemia category included both patients with currently high serum lipid levels and those with medical records confirming previous hyperlipidaemia.

#### *Hypertension*

The definition and the diagnosis of hypertension were based on the criteria proposed by the third joint national committee.[24] We collected both previous medical records of raised blood pressure and present systolic and diastolic blood pressure values. For the statistical evaluation of patients who were on antihypertensive drug treatment, we used the cate-

gorical data of occurrence or absence of hypertension as derived from previous medical records; for untreated patients, the diagnosis was derived from values recorded during the current hospital admission. The blood pressure values were the mean of three measurements made at 2–4 min intervals with a random zero sphygmomanometer on subjects at rest.

*Cigarette smoking*

We distinguished two groups of patients: smokers, consisting of current smokers and patients who had ceased smoking; and non-smokers, who were patients with no history of smoking. Evaluation of the number of cigarettes consumed per day was thought to be unreliable.

*Diabetes*

We recorded both insulin dependent and non-insulin-dependent (both obese and non-obese types) diabetes mellitus in accordance with standardised criteria.[25]

*Family history of coronary artery disease*

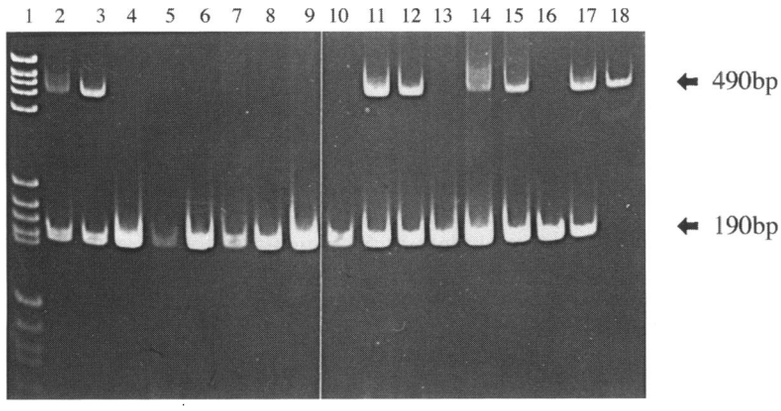
Since a positive family history is a predictor of myocardial infarction independently of other variables,[26] this risk factor was collected on the basis of documented medical records confirming the occurrence of ischaemic heart disease in collaterals and in parents.

*Obesity*

To determine obesity we analysed body weight relative to height in comparison with normal standards (as categorised by sex, age, and height). Obesity was defined as a body weight of 20% or more above the norm.[27]

GENOTYPE STUDY: DNA EXTRACTION

Genomic DNA was extracted from peripheral blood leucocytes using standard techniques.[28] DNA concentrations were measured by absorbance at 260 nm.




*Determination of ACE genotypes by polymerase chain reaction (PCR) amplification: 1·5% agarose gel stained with ethidium bromide and photographed under ultraviolet transillumination. The picture shows the three patterns of PCR products (*II, DD, *and* ID*) for the ACE gene insertion/deletion polymorphism. Lane 1: molecular weight markers (pBR322/HaeIII); lanes 2, 3, 11, 12, 14, 15, 17 contain PCR products from heterozygotes, with presence of both the 190* bp D *fragment and of the 490* bp I *fragment; lanes 4, 5, 6, 7, 8, 9, 10, 13, 16 contain* DD *homozygotes with only the 190* bp D *fragment; lane 18 contains* II *homozygote with only the 490 bp fragment.*

DETECTION OF INSERTION/DELETION POLYMORPHISM IN THE ACE GENE

This was done by polymerase chain reaction (PCR).[29] DNA from each sample (0·1 μg) was subjected to DNA amplification in 50 μl of reaction buffer: 50 mM potassium chloride, 10 mM tris-hydrochloride pH 8·3, 1·5 mM magnesium chloride, 20 nM of each deossinucleotide triphosphate, 50 pmol of each primer, and 0·5 U of AmpliTaq™ (Perkin-Elmer Cetus). Amplification was maintained for 30 cycles (denaturation 94°C for 1·20 min, annealing 58°C for 1·30 min, extension 72°C for 2·20 min). Reaction products were analysed on 5% polyacrylamide gel in tris-borate ethylenediaminetetraacetic acid buffer. The products were then stained with ethidium bromide and photographed for allele identification. To amplify the ACE gene polymorphic fragment from human genomic DNA, we used a 391 DNA synthesiser (Applied Biosystems) to synthesise two oligonucleotides (5′ CTG GAG ACC ACT CCC ATC ATT TCT 3′ and 5′ GAT GTG GCC ATC ACA TTG GTC AGA T 3′), as designed by Tiret and coworkers.[4] These oligonucleotides allow the detection of a 190 bp fragment in the absence of insertion and a 490 bp fragment in the presence of insertion in the ACE gene[4] (figure).

STATISTICAL METHODS

We estimated the allele frequencies in test and control groups using the gene counting method and compared them by the $\chi^2$ test, with the values predicted by the assumption of Hardy-Weinberg equilibrium in the sample.

Mean and standard deviation or frequency distribution were calculated for each of the given variables, that is, ACE genotype, myocardial infarction, hypertension, age, sex, hyperlipidaemia and cholesterol concentrations, triglyceride concentrations, cigarette smoking, diabetes mellitus, obesity, and a family history of ischaemic heart disease. The bivariate distribution of the recorded risk factors between the differing genotype groups was assessed by means of 2 × K tables. $\chi^2$ Tests were calculated. The association of ACE gene polymorphisms with coronary atherosclerosis, hypertension and myocardial infarction was first tested by means of 2 × 2 traditional table analysis: we calculated odds ratios (OR) (maximum likelihood estimation) along with their confidence intervals. Their significance was tested by means of $\chi^2$ test for OR = 1. Finally, to better explore these associations and, in particular, to evaluate the collective influence of several variables on the occurrence of coronary atherosclerosis and myocardial infarction, we used logistic regression to identify appropriate models.[30] This multivariate technique allows us to assess both the individual and the joint influence of several factors that may affect a binary response variable (absence or occurrence of coronary atherosclerosis; absence or occurrence of myocardial infarction). We performed marginal and conditional tests which respectively correspond to univariate and multivariate analysis, in order to detect the best predictors for the model. We

*Table 1 Clinical data and distribution of risk factors in patients with (+) and without (−) coronary atherosclerotic disease (CAD)*

| | *Number of observations* | *CAD+ (n = 255)* | *CAD− (n = 133)* | *P value* |
|---|---|---|---|---|
| Age, mean (SD) | 388 | 61·3 (10·91) | 59·31 (15·67) | NS* |
| Gender: M/F ratio | 388 | 203/52 (3·9) | 78/55 (1·4) | 0·0001 |
| Hyperlipidaemia[a] | 359[c] | 117 (48%) | 44 (40%) | NS† |
| Cholesterol, mg/dl mean: (SD) | 388 | 219 (46) | 205 (53) | NS* |
| Triglyceride, mg/dl mean: (SD) | 388 | 153 (111) | 117 (60) | 0·002* |
| Hypertension[b] | 388 | 93 (37%) | 36 (27%) | NS† |
| Family history | 341[c] | 120 (52%) | 50 (46%) | NS† |
| Diabetes mellitus | 388 | 32 (13%) | 6 (4·5%) | 0·01077† |
| Present and previous smoking | 343[c] | 172 (73%) | 45 (41%) | 0·00000† |
| Obesity | 388 | 17 (7%) | 7 (5%) | NS† |

[a] Proven knowledge of lipid status; [b] based on present values in non-treated patients and on history in treated patients; [c]No of observations for each variable may differ from total no of observations because of missing or unreliable data.
†Pearson $\chi^2$; *unpaired $t$ test.

used the models to test the following covariates in addition to the genotype: age, sex, cholesterol and triglyceride concentrations, family history of coronary heart disease, smoking status, diabetes, hypertension, and obesity. The models finally selected included all the variables that appeared to modify the model conditionally. Computation was performed by EGRET®.[31]

## Results

### CORONARY ATHEROSCLEROSIS

We identified 255 patients with coronary atherosclerosis and 133 patients with angiographically normal coronary arteries. Clinical data on age, sex, and risk factors are summarised in table 1. In the coronary atherosclerosis group, the incidence of males, smokers, and patients with diabetes and hypertrigliceridaemia was significantly higher than in the group without coronary atherosclerosis. Mean age and cholesterol concentrations, as well as the percentage of patients with obesity, a family history of coronary artery disease, hypertension, and hyperlipidaemia, were higher in the group of patients with coronary atherosclerosis than in patients without coronary atherosclerosis, but the difference was not significant.

### ALLELE FREQUENCY IN HEALTHY POPULATION AND IN THE STUDY GROUP

The distribution of ACE genotypes in the test and control groups was in Hardy-Weinberg equilibrium. The respective frequencies for *I* and *D* alleles in our clinically healthy population with unknown coronary artery status (n = 290) were 0·37 (*I*) (95% confidence interval (CI): 0·32–0·42) and 0·63 (*D*) (95% CI: 0·58–0·68), and were virtually identical to the frequencies observed in our overall study population (n = 388), at 0·36 (*I*) (95% CI: 0·31–0·41) and 0·64 (*D*) (95% CI: 0·59–0·69) respectively, as were the the frequencies of the three genotypes: *II*, 16% (n = 46) *v* 14% (n = 53); *ID*, 43% (n = 124) *v* 44% (n = 172); *DD*, 41% (n = 120) *v* 42% (n = 163).

*D* allele frequency in the subgroup of patients with coronary atherosclerosis (n = 255 of 388) was significantly higher than in patients without coronary atherosclerosis (P < 0·001) (table 2A). The opposite was true for the *I* allele.

### ACE GENOTYPE FREQUENCIES IN PATIENTS WITH AND WITHOUT CORONARY ATHEROSCLEROSIS

ACE genotypes were differently distributed in the two groups. The *ID* and *DD* genotypes were higher in the coronary atherosclerosis group than in the normal coronary artery group: in particular, the percentage of homozygotes (*DD*) in the coronary atherosclerosis group was about twice that of the normal coronary artery group (table 2B). The occurrence of coronary atherosclerosis was associated with the genotype, with an odds ratio of 5·78 for the *DD* pattern and of 2·39 for the *ID* pattern, in comparison with the *II* pattern (table 3). Differing ORs were estimated in males and females (table 3), because of the effect modifying role of sex; this did not, however, confound the association.

### ACE GENOTYPES IN PATIENTS WITH MYOCARDIAL INFARCTION

The frequency of ACE genotypes differed between patients with (n = 154) and without (n = 234) myocardial infarction. *DD* polymorphism was higher in patients with myocardial infarction than in those without (table 2B). The occurrence of acute or previous myocardial infarction was associated with the *DD* ACE genotype, with an OR of 2·56. The OR for the *ID* genotype in comparison with that

*Table 2 Distribution of ACE allele frequency (A) and genotypes (B) according to occurrence (+) or absence (−) of coronary atherosclerosis (CAD), myocardial infarction (MI), and hypertension (HT)*

| *(A) Allele frequencies* | *I* | *% (95% CI)* | *D* | *% (95% CI)* |
|---|---|---|---|---|
| CAD+ (510) | 147 | 28·8 (26·8–30·8) | 363 | 71·2 (69·2–73·2) |
| CAD− (266) | 131 | 49·2 (46·1–52·3) | 135 | 50·8 (47·7–50·5) |
| MI+ (308) | 93 | 30·2 (27·6–32·8) | 215 | 69·8 (67·2–72·4) |
| MI− (468) | 185 | 39·5 (37·2–41·8) | 283 | 60·5 (58·2–62·8) |
| HT+ (258) | 86 | 33·3 (30·4–36·2) | 172 | 66·7 (63·8–69·6) |
| HT− (518) | 192 | 37·1 (35·0–39·2) | 326 | 62·9 (60·8–65·0) |

| *(B) ACE genotypes* | *II (n = 53)* | | *ID (n = 172)* | | *DD (n = 163)* | |
|---|---|---|---|---|---|---|
| | *n* | *% (95% CI)* | *n* | *% (95% CI)* | *n* | *% (95% CI)* |
| CAD+ (n = 255) | 21 | 8·2 (4·8–11·6) | 105 | 41·2 (35·2–47·2) | 129 | 50·6 (44·5–56·7) |
| CAD− (n = 133) | 32 | 24·1 (16·8–31·3) | 67 | 50·4 (41·9–58·8) | 34 | 25·5 (18·0–32·9) |
| MI+ (n = 154) | 13 | 8·4 (4·1–12·7) | 67 | 43·5 (35·7–51·3) | 74 | 48·1 (40·3–55·9) |
| MI− (n = 234) | 40 | 17·1 (12·2–22·0) | 105 | 44·9 (38·6–51·2) | 89 | 38 (31·7–44·3) |
| HT+ (n = 129) | 13 | 10·1 (5·0–15·2) | 60 | 46·5 (37·9–55·1) | 56 | 43·4 (34·8–52·0) |
| HT− (n = 259) | 40 | 15·4 (11·1–19·7) | 112 | 43·3 (37·2–49·4) | 107 | 41·3 (35·2–47·3) |

CI, confidence interval.

*Table 3 Coronary atherosclerosis, myocardial infarction, and hypertension: odds ratios (OR) and confidence intervals (CI) of the DD and ID ACE genotypes in comparison with the II pattern*

| | Genotype | OR | CI (95% Cornfield) | P value |
|---|---|---|---|---|
| Coronary atherosclerosis: | | | | |
| Test for OR = 1; $\chi^2$ = 30·64; | | | | |
| P < 0·001 | *DD/II* | 5·78 | 2·82–11·93 | <0·001 |
| | *ID/II* | 2·39 | 1·22–4·71 | 0·006 |
| Stratified by sex (all cases) | | | | |
| Test for OR = 1; $\chi^2$ = 27·43; | | | | |
| P < 0·001 | *DD/II* | 5·63 | 2·63–11·62 | <0·001 |
| | *ID/II* | 2·37 | 1·17–4·68 | 0·009 |
| Males | | | | |
| Test for OR = 1; $\chi^2$ = 14·91; | | | | |
| P < 0·001 | *DD/II* | 4·20 | 1·72–10·30 | <0·001 |
| | *ID/II* | 1·76 | 0·76–4·07 | 0·151 |
| Females | | | | |
| Test for OR = 1; $\chi^2$ = 13·38; | | | | |
| P < 0·001 | *DD/II* | 11·11 | 2·35–59·94 | <0·001 |
| | *ID/II* | 4·92 | 1·13–24·44 | 0·015 |
| Mycardial infarction | | | | |
| Test for OR = 1; $\chi^2$ = 7·33; | | | | |
| P = 0·026 | *DD/II* | 2·56 | 1·21–5·47 | 0·007 |
| | *ID/II* | 1·96 | 0·93–4·19 | 0·056 |
| Stratified by coronary atherosclerosis | | | | |
| Test for OR = 1; $\chi^2$ = 0·91; | | | | |
| P < 0·635 | *DD/II* | 0·83 | 0·29–2·33 | 0·697 |
| | *ID/II* | 1·12 | 0·40–3·13 | 0·814 |
| Hypertension | | | | |
| Test for OR = 1; $\chi^2$ = 2·11; | | | | |
| P = 0·349 | *DD/II* | 1·61 | 0·76–3·47 | 0·184 |
| | *ID/II* | 1·65 | 0·78–3·53 | 0·160 |

for the *II* (table 3) was 1·96. The association between genotype and myocardial infarction was further tested in the coronary atherosclerosis group and was not significant ($\chi^2$ 0·762, P = 0·68). In addition, stratified 2 × K analysis showed coronary atherosclerosis to be a confounder of the relationship between genotype and myocardial infarction. The latter lost its significance after controlling for atherosclerosis (table 3): the association of genotype with myocardial infarction seems to be entirely justified by the presence of coronary atherosclerosis.

*Table 4 Distribution of the tested categorical variables in the ACE genotype groups*

| | *II* (n = 53) | *ID* (n = 172) | *DD* (n = 163) | *P value* |
|---|---|---|---|---|
| Sex (n = 388) | | | | 0·13 |
| males (n = 281) | 34 | 122 | 125 | |
| females (n = 107) | 19 | 50 | 38 | |
| Family history of IHD (n = 341) | | | | 0·48 |
| + | 22 | 75 | 73 | |
| − | 28 | 78 | 65 | |
| Hypertension (n = 388) | | | | 0·30 |
| + | 13 | 61 | 56 | |
| − | 40 | 112 | 106 | |
| Diabetes (n = 388) | | | | 0·27 |
| + | 3 | 21 | 14 | |
| − | 50 | 152 | 148 | |
| High cholesterol (n = 359) | | | | 0·16 |
| + | 28 | 71 | 62 | |
| − | 21 | 93 | 84 | |
| Obesity (n = 388) | | | | 0·18 |
| + | 6 | 11 | 7 | |
| − | 47 | 162 | 155 | |
| Smoker (n = 343) | | | | 0·15 |
| + | 27 | 86 | 74 | |
| − | 20 | 70 | 66 | |

n, number of reliable observations available; IHD, ischaemic heart disease.

*Table 5 Final logistic model for coronary atherosclerosis: regression coefficients with their significance and their logistic odds ratios and confidence intervals (CI)*

| *Variable* | *Coefficient* | *P value* | *Odds ratio* | *95% CI* |
|---|---|---|---|---|
| %GM | − 3·221 | < 0·001 | 0·03993 | 0·007597–0·2098 |
| Sex F *v* M | − 1·015 | 0·005 | 0·3624 | 0·1788–0·7347 |
| Age | 0·03259 | 0·004 | 1·033 | 1·010–1·056 |
| Genotype *DD** | 1·935 | < 0·001 | 6·925 | 3·094–15·50 |
| Genotype *ID** | 1·060 | 0·005 | 2·886 | 1·373–6·067 |
| Hypertension | 0·6794 | 0·027 | 1·973 | 1·079–3·607 |
| High cholesterol | 0·7900 | 0·006 | 2·203 | 1·254–3·871 |
| Smoking | 1·051 | 0·001 | 2·861 | 1·520–5·382 |

%GM, grand mean; F, female; M, male.
*Odds ratios are computed respect to genotype *II*.

ACE GENOTYPES IN PATIENTS WITH HYPERTENSION

The frequency of systemic hypertension was higher in patients with than without the deletion allele (*DD* and *ID*) (table 2B). However, hypertension was unrelated to ACE genotypes, with respective ORs of 1·61 for the *DD* pattern and 1·65 for the *ID* pattern in comparison with the *II* genotype (table 3).

RISK FACTORS AND ACE GENOTYPE

Given the association of ACE *ID* polymorphism with coronary atherosclerosis, we evaluated the distribution in the three genotypes of a series of potentially disease related variables, namely, age, sex, family history of ischaemic heart disease, hypertension, diabetes, hypercholesterolaemia, obesity, and smoking. As shown in table 4, a similar distribution was observed in the three genotypes for all the categorical covariates.

LOGISTIC MODELS

We performed logistic regression to test the association of the selected risk factors with atherosclerosis and myocardial infarction.

*Coronary atherosclerosis*

In our final logistic model, age, sex, ACE genotypes *DD* and *ID*, hypertension, hypercholesterolaemia, and smoking appeared to be independent predictors of coronary atherosclerosis. Table 5 gives the regression coefficients together with their significance and their logistic ORs (with 95% confidence intervals). The presence of the shorter allele (*D*) represents the most important risk factor for coronary atherosclerosis. The occurrence of a *DD* genotype multiplies the risk by 7 in comparison with *II* pattern, whereas the *ID* genotype multiplies the risk by 3. As shown in table 5, the strongest association for coronary atherosclerosis was with *DD* and *ID* ACE genotypes, sex, and smoking.

*Myocardial infarction*

In our logistic model, sex, ACE genotypes *DD* and *ID*, and high cholesterol concentrations appeared to be independent predictors of myocardial infarction. Table 6 gives the logistic regression coefficients, along with their significance and their logistic ORs (with 95% confidence intervals). The *DD* genotype represented a lower risk factor for myocardial infarction than for atherosclerosis: it actually multiplied the risk by 3·5 in comparison with the *II* pattern. Moreover, the *ID* genotype, which had a borderline significant association with infarction in traditional 2 × 2 analysis (P = 0·056), proved in the final logistic model (after controlling for the other covariates) to multiply the risk by 2·7 in comparison with the *II* pattern. In addition, sex (male) and high cholesterol levels both appeared to increase the risk of myocardial infarction. None of the other variables tested in the model was found to predict myocardial infarction.

*Table 6 Final logistic model for myocardial infarction: regression coefficients with their significance and their logistic odds ratios and confidence intervals (CI)*

| *Variable* | *Coefficient* | *P-value* | *Odds ratio* | *95% CI* |
|---|---|---|---|---|
| %GM | − 1·316 | 0·079 | 0·2683 | 0·0619–1·162 |
| Sex: F *v* M | − 1·212 | < 0·001 | 0·2975 | 0·1664–0·5320 |
| Age | 0·0018 | 0·859 | 1·002 | 0·9821–1·022 |
| Genotype *DD** | 1·239 | 0·002 | 3·450 | 1·559–7·639 |
| Genotype *ID** | 1·003 | 0·012 | 2·727 | 1·245–5·972 |
| High cholesterol | 0·6193 | 0·011 | 1·858 | 1·152–2·997 |

%GM, grand mean; F, female; M, male.
*Odds ratios are computed respect to genotype *II*.

ACE GENOTYPES IN LOW RISK PATIENTS WITH AND WITHOUT CORONARY ATHEROSCLEROSIS AND MYOCARDIAL INFARCTION

Of the 388 patients who constituted the test group, 87 were free of all the tested risk factors. In this subgroup there was no significant difference in ACE genotype distribution between males and females (P = 0·425). In the 87 patients, we found a significant association between ACE genotype and the occurrence or absence of coronary atherosclerosis (P = 0·0008). ORs were computed to measure the association between genotype (*II* + *ID* and *DD*) and coronary atherosclerosis: $OR_{II + ID/DD}$ was higher in the low risk group than in the high risk group at 5·46 (95% CI: 1·38–4·37) *v* 2·45 (95% CI: 1·38–4·37). In the same subpopulation, the distribution of the three genotypes did not differ significantly between patients with and without infarction (P = 0·232).

## Discussion

Our study shows that the deletion allele in the ACE gene is independently associated with coronary atherosclerosis and with myocardial infarction: compared to the absence of the shorter allele, the occurrence of the *DD* genotype multiplies the risk of coronary atherosclerosis by 7 and that of myocardial infarction by 3·5. The *DD* genotype seems to act independently of other known risk factors, which usually interact cumulatively to create high risk individuals. In our logistic model, seven independent variables were associated with the occurrence of coronary atherosclerosis: sex, age, *DD* and *ID* genotypes, hypertension, hypercholesterolaemia, and smoking. In contrast diabetes, family history of ischaemic heart disease, and triglyceride concentrations were not independently associated with the disease, even though the frequency of diabetes and the mean triglyceride concentrations were higher in the atherosclerosis than in the normal coronary artery group. It should be pointed out that dyslipidaemia and hypertension constitute risk factors that may be widely reversed by treatment and by diet or lifestyle modifications, and that it is very uncommon, in our country, to find patients in their sixties who are not aware (and therefore in control) of their own lipids or blood pressure. The model shows that *DD* polymorphism in this type of patient is the strongest risk factor for coronary atherosclerosis. Similarly, though to a lesser extent, the *ID* pattern accounts for a threefold increase in risk. The association of the *D* allele with coronary atherosclerosis was confirmed in the subgroup of patients without any known risk factor. Although our model needs to be validated in further independent series, the results in our population are quite suggestive of a role of the ACE locus in the pathogenesis of coronary atherosclerosis. Our data on coronary atherosclerosis agree with previously reported data in French,[8] Japanese,[9] and Welsh[10] coronary patients, but partly contrast with those reported in two separate series of US male cases: the first study, exclusively involving white males, showed that the ACE genotype was only associated with myocardial infarction, and not with atherosclerosis;[6] the second, a prospective case-control study involving a predominantly white population of male US physicians, failed to detect any association between the *D* allele and incidence of either myocardial infarction or other clinical manifestation of ischaemic heart disease.[13] These discrepancies may have several explanations: (1) the appropriateness of control cases to the aim of the studies (healthy blood donors with unknown coronary artery status *v* patients with non-ischaemic heart disease); (2) the variable allele frequencies in the ethnic groups tested to date; our allele frequency is similar to that of the Lille subgroup (ECTIM study)[5] and to a lesser extent to that of a Welsh population,[10] but differs slightly from that reported in other Europe and US populations,[5 6 11 13] and even more so from that reported in Chinese and Japanese populations.[9 32 33] The ethnic difference hypothesis deserves further investigation.

The reported association between ACE gene deletion polymorphism and myocardial infarction[5 6] is confirmed in our study, which shows a significantly higher frequency of infarction in patients with the *DD* and *ID* ACE genotypes than was found in *II* genotype patients. *D* allele related risk is higher for coronary atherosclerosis than for myocardial infarction, which represents a subset of the clinically overt tip of the iceberg of coronary atherosclerosis. The lack of association between myocardial infarction and most of the other tested risk factors that influence the occurrence of coronary atherosclerosis suggests that the true determinants of infarction are different from those that cause coronary atherosclerosis. Myocardial infarction is caused by occlusive coronary thrombosis, which occurs in most cases over deeply ulcerated atherosclerotic plaques. This event is relatively rare with respect to the spread and the frequency of coronary atherosclerosis, and is potentially related to factors that have not even been tested in our study, given that they are still largely unknown and that they seem to be resident (plaque related) rather than systemically detectable. Alternatively, the link between infarction (caused by coronary plaque ulceration and thrombosis) and ACE genotype might lie in the concentration of activated inflammatory cells in those plaque caps where inflammatory cells promote plaque ulceration

or erosion,[34 35] given that ACE genotypes highly influence T lymphocytes and macrophage ACE concentrations.[36] If this is true, perhaps ACE exerts proaggregant or procoagulant local effects. This possibility is indirectly supported by the evidence that patients treated with ACE inhibitors for essential hypertension display reductions in platelet aggregation in vitro.[37] Islim and coworkers showed that plasma $\beta$ thromboglobulin, which is a marker of in vivo platelet activation, is significantly reduced by treatment in patients with hypertension.[38] In the SAVE and in the SOLVD trials, the long term administration of an ACE inhibitor was associated with reductions in recurrent myocardial infarction and in mortality.[39 40] However, the precise mechanism by which ACE might influence acute coronary occlusion is still a matter of hypothesis.

So far as ACE genotype and hypertension is concerned, the present study joins Cambien and co-workers,[5] Jeunemaitre *et al*,[41] and Higaschimori *et al* (who studied a Japanese population),[32] in not detecting any association between ACE gene deletion polymorphism and hypertension. It is likely that genes other than ACE exert their control on pressure levels. Very recently, the locus for angiotensinogen (the third non-enzymatic component of the renin-angiotensin cascade) has been directly implicated in hypertension. Jeunemartre *et al* showed that there is an excessive sharing of angiotensinogen alleles between affected hypertensive sibships, and that some angiotensin molecular variants, which are related to raised plasma angiotensin concentrations, are correlated with hypertension.[42]

## LIMITS OF THE STUDY

A possible limit of the present study derives from the use (as control for the group with coronary atherosclerosis) of patients with cardiac diseases in which coronary angiography showed a normal coronary tree, rather than of healthy patients with normal coronary arteries. This limit is obviously intrinsic to this type of study, given the impossibility of our knowing the status of coronary arteries in healthy subjects. To prevent population related bias and to be sure that the genotype frequencies in our test group were comparable with those that of the general population, we studied 290 healthy blood donors: the similarity of *I* and *D* allele frequencies between the two groups indicates that allele frequency distribution is analogous in the overall test group and that only the occurrence of coronary atherosclerosis allows us to recognise differences in *I* and *D* allele distribution. It is likely that our control group of clinically healthy subjects (their mean age was lower than that of the study group) included cases with silent coronary artery disease, as well as cases who will in future develop ischaemic disease and myocardial infarction. It should also be noted that not only coronary atherosclerosis[43] but also myocardial scars, documenting previous infarctions, may be occasional findings in necropsy studies.[44]

## CONCLUSION

Our study identifies ACE gene deletion as strongly and independently associated with coronary atherosclerosis. The opposite condition in patients with angiographically normal coronary arteries gives strength to previous data in French, Japanese, and Welsh coronary patient series, in which the coronary arteries of control group subjects were supposed to be normal on the basis of clinical data.[7] Furthermore, our results confirm in white Italian patients what Cambien *et al*[5] and Ludwig *et al*[6] found in European and US populations, namely an association between the *D* allele and myocardial infarction which, in our series, was less than the association between the *D* allele and coronary atherosclerosis, but was nonetheless significant.

We thank: Dr S Surrey, The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA, USA, for the useful revision of our study, and Prof C Berzuini, Department of Informatics, University of Pavia, Italy. The study was supported by Health Ministry grants "Ricerche Finalizzate: Patologia Cardiovascolare" (1994–5) and by IRCCS Policlinico S Matteo, Pavia.

1 Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corval P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 1990; **86**:1343–6.
2 Dux S, Aron N, Boner G, Carmel A, Taron A, Rosenfeld JB. Serum angiotensin-converting enzyme activity in normal adults and patients with different types of hypertension. *Isr J Med Sci* 1984;**20**:1138–41.
3 Alhenc-Gelas F, Richard J, Courbon D, Warnet JM, Corval P. Distribution of plasma angiotensin I-converting enzyme levels in healthy men: relationship to environmental and hormonal parameters. *J Lab Clin Med* 1991; **117**:33–9.
4 Tiret L, Rigat B, Visvikis S, Breda C, Corvol P, Cambien F, *et al*. Evidence, from combined segregation and linkage analysis, that a variant of the angiotensin I-converting enzyme (ACE) gene controls plasma ACE levels. *Am J Hum Genet* 1992;**51**:197–205.
5 Cambien F, Poirier O, Lecerf L, Evans A, Cambou JP, Arveiler D, *et al*. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature* 1992;**359**:641–4.
6 Ludwig E, Corneli PS, Anderson JL, Marshall HW, Lalouel JM, Ward RH. Angiotensin-converting enzyme gene polymorphism is associated with myocardial infarction but not with development of coronary stenosis. *Circulation* 1995;**91**:2120–4.
7 Tiret L, Kee F, Poirier O, Nicaud V, Lecerf L, Evans A, *et al*. Deletion polymorphism in angiotensin-converting enzyme gene associated with parental history of myocardial infarction. *Lancet* 1993;**341**:991–2.
8 Ruiz J, Blanché H, Cohen N, Velho G, Cambien F, Cohen D, *et al*. Insertion/deletion polymorphism of the angiotensin-converting enzyme gene is strongly associated with coronary heart disease in non-insulin-dependent diabetes mellitus. *Proc Natl Acad Sci USA* 1994; **91**:3662–5.
9 Nakai K, Itoh C, Miura Y, Musha T, Itoh T, Miyakawa T, *et al*. Deletion polymorphism of the angiotensin I converting enzyme gene is associated with serum ACE concentration and increased risk for CAD in the Japanese. *Circulation* 1994;**90**:2199–202.
10 Mattu RK, Needham EWA, Galton DJ, Frangos E, Clark AJL, Caulfield M. A DNA variant at the angiotensin-converting enzyme gene locus associated with coronary artery disease in the Caerphilly Heart Study. *Circulation* 1995;**91**:270–4.
11 Raynolds MV, Bristow MR, Bush EW, Abraham W, Lowes BD, Zisman LS, *et al*. Angiotensin-converting enzyme DD genotype in patients with ischemic or idiopathic dilated cardiomyopathy. *Lancet* 1993;**342**:1073–5.
12 Arbustini E, Pozzi R, Grasso M, Gavazzi A, Diegoli M, Bramerio M, *et al*. Pathologic substrates and clinical correlates of coronary artery disease and chronic congestive heart failure requiring cardiac transplantation. *Coronary Artery Disease* 1991;**2**:605–12.
13 Lindpaintner K, Pfeffer MA, Kreutz R, Stampfer MJ, Grodstein F, LaMotte F, *et al*. A prospective evaluation of an angiotensin-converting-enzyme gene polymorphism and the risk of ischemic heart disease. *N Engl J Med* 1995;**332**:706–11.
14 Braunwald E, ed. *Heart Disease* 3rd ed. Philadelphia: WB Saunders, 1988.
15 Sones FM. Indications and value of coronary arteriography. *Circulation* 1972;**46**:1155–70.

16 Abrams HL. *Coronary arteriography. A practical approach.* Boston: Little Brown, 1983.
17 Betriu A, Castaner A, Sanz GA, Pare JC, Roig E, Coll S, *et al.* Angiographic finding 1 month after myocardial infarction: a prospective study of 259 survivors. *Circulation* 1982;**65**:1099–105.
18 Miller RD, Burchel HB, Edwards JE. Myocardial infarction with and without acute coronary occlusion: a pathologic study. *Arch Intern Med* 1951;**88**:597–601.
19 Wei JY, Bulkley BH. Myocardial infarction before age 36 years in women: predominance of apparent nonatherosclerotic events. *Am Heart J* 1982;**104**:561–6.
20 Spodick DH. Inflammation and the onset of myocardial infarction. *Ann Intern Med* 1985;**102**:699–702.
21 Homcy CJ, Liberthson RR, Fallon JT, Gross S, Miller LM. Ischemic heart disease in systemic lupus erythematosus in the young patient; Report of six cases. *Am J Cardiol* 1982;**49**:478–84.
22 Consensus Conference Statement: Lowering Blood Cholesterol to prevent heart disease. *JAMA* 1985;**253**: 2080–6.
23 Study Group European Atherosclerosis Society Strategies for the prevention of coronary heart disease. A policy statement of the European Atherosclerosis Society. *Eur Heart J* 1987;**8**:77–88.
24 Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure. The 1984 report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure. *Arch Intern Med* 1984;**144**:1045–57.
25 World Health Organisation: *Diabetes Mellitus* (Tech Rep Ser) Geneva: WHO, 1985:727.
26 Friedlander Y, Kark JD, Stein Y. Family history of myocardial infarction as an independent risk factor for coronary heart disease. *Br Heart J* 1985;**53**:382–7.
27 Bray GA. *Obesity in America.* Washington DC: US Dept of Health Education and Welfare, Publication No (NIH) 79–359, 1979.
28 Marcadet A, O'Connell P, Cohen D. Standardized Southern blot workshop technique. In: Dupont, ed. *Histocompatibility Testing* Vol 1B. New York: Springer-Verlag 1987:553–60.
29 Saiki RK, Gelfand DH, Stoffel S, Sharf SJ, Higuchi R, Horn GT, *et al.* Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 1988;**239**:487–91.
30 Benedetti JK, Brown MB. Strategies for the selection of log-linear models. *Biometrics* 1978;**34**:680–6.
31 EGRET® *Reference manual.* Statistics and Epidemiology Research Corporation and Cytel Software Corporation. Copyright 1985–91.
32 Higashimori K, Zhao Y, Higaki J, Kamitani A, Katsuya T, Nakura J, *et al.* Association analysis of a polymorphism of the angiotensin converting enzyme gene with essential hypertension in the Japanese population. *Biochem Biophys Res Commun* 1993;**191**:399–404.
33 Lee EJ. Population genetics of the angiotensin-converting enzyme in Chinese. *Br J Clin Pharmacol* 1994;**37**:212–14.
34 Van der Wal AC, Das PK, Bentz-van-de Berg D, Van der Loos CM, Becker AE. Atherosclerotic lesions in humans. In situ immunophenotypical analysis suggesting an immune mediated response. *Lab Invest* 1989;**61**:166–70.
35 Arbustini E, De Servi S, Bramucci E, Porcu E, Costante AM, Grasso M, *et al.* Comparison of coronary lesions obtained by directional coronary atherectomy in unstable angina, stable angina, and restenosis after either atherectomy or angioplasty. *Am J Cardiol* 1995;**75**:675–82.
36 Costerousse O, Allegrini J, Lopez M, Alhenc-Gelas F. Angiotensin I-converting enzyme in human circulating mononuclear cells: genetic polymorphism of expression in T-lymphocytes. *Biochem J* 1993;**290**:33–40.
37 James IM, Dickinson EJ, Burgoyne W, Jeremy JY, Barradar MA, Mikhailidis DP, *et al.* Treatment of hypertension with captopril: preservation of regional blood flow and reduced platelet aggregation. *J Hum Hypertens* 1988;**2**: 21–5.
38 Islim IF, Beevers DG, Bareford D. The effect of antihypertensive drugs on in vivo platelet activity in essential hypertension. *J Hypertens* 1992;**10**:379–83.
39 Pfeffer MA, Braunwald E, Moyét LA, Basta L, Brown EJ, Cuddy TE, *et al.* Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction. *N Engl J Med* 1992;**327**: 669–77.
40 The SOLVD Investigators. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. *N Engl J Med* 1991;**325**: 293–302.
41 Jeunemaitre X, Lifton RP, Hunt SC, Williams RR, Lalouel JM. Absence of linkage between the angiotensin-converting enzyme and human essential hypertension. *Nature Genet* 1992;**1**:72–5.
42 Jeunemaitre X, Soubrier F, Kotelevtsev YV, Lifton RP, Williams CS, Charru A, *et al.* Molecular bases of human hypertension: role of angiotensinogen. *Cell* 1992;**71**: 169–180.
43 Arbustini E, Grasso M, Diegoli M, Morbini P, Aguzzi A, Fasani R, *et al.* Coronary thrombosis in non-cardiac death. *Coronary Artery Dis* 1993;**4**:751–9.
44 Kannel WB, Abbott R. Incidence and prognosis of unrecognized myocardial infarction. An update on the Framingham Study. *N Engl J Med* 1984;**311**:1144–7.