

## A PROSPECTIVE EVALUATION OF AN ANGIOTENSIN-CONVERTING-ENZYME GENE POLYMORPHISM AND THE RISK OF ISCHEMIC HEART DISEASE

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**Abstract Background.** In a previous study, men with a history of myocardial infarction were found to have an increased prevalence of homozygosity for the deletional allele (*D*) of the angiotensin-converting-enzyme (*ACE*) gene. The *D* allele is associated with higher levels of *ACE*, which may predispose a person to ischemic heart disease. We investigated the association between the *ACE* genotype and the incidence of myocardial infarction, as well as other manifestations of ischemic heart disease, in a large, prospective cohort of U.S. male physicians.

**Methods.** In the Physicians' Health Study, ischemic heart disease as defined by angina, coronary revascularization, or myocardial infarction developed in 1250 men by 1992. They were matched with 2340 controls according to age and smoking history. Zygosity for the deletion-insertion (*D-I*) polymorphism of the *ACE* gene was determined by an assay based on the polymerase chain reaction. Data were analyzed for both matched pairs and

unmatched samples, with adjustment for the effects of known or suspected risk factors by conditional and non-conditional logistic regression, respectively.

**Results.** The *ACE* genotype was not associated with the occurrence of either ischemic heart disease or myocardial infarction. The adjusted relative risk associated with the *D* allele was 1.07 (95 percent confidence interval, 0.96 to 1.19;  $P=0.24$ ) for ischemic heart disease and 1.05 (95 percent confidence interval, 0.89 to 1.25;  $P=0.56$ ) for myocardial infarction, if an additive mode of inheritance is assumed. Additional analyses assuming dominant and recessive effects of the *D* allele also failed to show any association, as did the examination of low-risk subgroups.

**Conclusions.** In a large, prospectively followed population of U.S. male physicians, the presence of the *D* allele of the *ACE* gene conferred no appreciable increase in the risk of ischemic heart disease or myocardial infarction. (N Engl J Med 1995;332:706-11.)

**I**SCHEMIC heart disease is a multifactorial disease, influenced by environmental and genetic factors. Although the recognition of a number of environmental risk factors has led to important advances in the prevention and treatment of the disease, our knowledge of its heritability, except in the case of uncommon monogenic disorders,<sup>1</sup> is limited to the predictive importance of a positive family history<sup>2,3</sup> and to observations of familial aggregation.<sup>4,5</sup> Thus, a recently reported association between a polymorphism of the angiotensin-converting-enzyme (*ACE*) gene and the occurrence of myocardial infarction generated great interest.<sup>6</sup>

The *ACE* gene (encoding kininase II, EC 3.4.15.1)<sup>7,8</sup> contains a polymorphism based on the presence (insertion [*I*]) or absence (deletion [*D*]) within an intron of a 287-base-pair (bp) nonsense DNA domain,<sup>9,10</sup> resulting in three genotypes (*DD* and *II* homozygotes, and *DI* heterozygotes). In a retrospective study in France and Ulster, men with the *DD* genotype were found to have 1.34 times the risk of myocardial infarction found in those with the *DI* or *II* genotype.<sup>6</sup> Moreover, in a "low risk" subgroup of these men, those with the *DD* genotype had an even higher risk of myocardial infarction (odds ratio, 3.2).

Several biologic actions of *ACE* could be involved in

the pathogenesis of ischemic heart disease: the activation of angiotensin I and the inactivation of bradykinin potentially result in decreased tissue perfusion<sup>11-13</sup>; angiotensin-induced stimulation of plasminogen-activator inhibitor type 1<sup>14</sup> may foster the formation of occlusive coronary thrombi; and angiotensin-mediated promotion of growth may be involved in the pathogenesis of cardiac hypertrophy and ventricular remodeling.<sup>15-17</sup> The observed codominant association between the *D-I* polymorphism and plasma *ACE* activity (with values in people with the *DD* genotype about double the values in those with *II*, and intermediate values found in those with *DI*)<sup>10,18,19</sup> could be consistent with the reported increase in cardiovascular risk associated with the *DD* genotype.

Beyond the potential importance of the polymorphism as a predictor of risk, the recognition of a genetic variant of the *ACE* gene as a pathogenetic factor in myocardial infarction is of particular interest because of the ready availability of target-specific pharmacologic agents in the form of *ACE* inhibitors. Clearly, the clinical implications of this finding would be even more far-reaching if the *ACE* genotype not only was associated with the occurrence of myocardial infarction, but also represented a modifiable risk factor for ischemic heart disease. The potential importance of the finding mandates that it be based on investigational designs that meet rigid epidemiologic and molecular genetic standards. We therefore conducted a large-scale, prospective study to address this question.

### METHODS

#### Study Population

From the Physicians' Health Study, 1250 patients with ischemic heart disease reported by late 1992 and 2340 controls were identified among those for whom blood samples were available. The Physicians'

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Health Study is a randomized trial of aspirin and beta carotene, initiated in 1982 in 22,071 male, predominantly white, U.S. physicians 40 to 84 years of age at study entry.<sup>20,21</sup> Men with a history of angina, myocardial infarction, stroke, transient ischemic attacks, or cancer were excluded from the study.

Before randomization, the physicians received blood-collection kits and were asked to submit EDTA-anticoagulated blood samples for storage at  $-80^{\circ}\text{C}$ , a request with which 14,916 participants complied. Follow-up questionnaires were sent 6 and 12 months after randomization, and yearly thereafter, to obtain updated information on exposures and newly diagnosed disease. Whenever a myocardial infarction was reported, the medical records of the patient were reviewed by an end-points committee of physicians. Cases of myocardial infarction were confirmed if they met the World Health Organization criteria in terms of symptoms, enzyme elevations, or electrocardiographic changes. Electrocardiographic changes consistent with infarction that were discovered on routine examination were not included because the date of their occurrence could not be ascertained. Physicians reporting angina received a questionnaire on which they were asked for further details providing evidence of the diagnosis. The performance of coronary bypass surgery or angioplasty was ascertained from the patients' reports. Causes of death were ascertained from medical records, autopsy results, death certificates, and details of the circumstances of death. Deaths were classified as resulting from coronary heart disease on the basis of these records. Sudden death was not attributed to ischemic heart disease unless there was additional supportive evidence.

In completing the questionnaire, the physicians provided information on a variety of characteristics relevant to the assessment of coronary risk, including diabetes mellitus, hypertension, cigarette smoking, body weight, height, hypercholesterolemia, physical activity, alcohol intake, and family history of cardiovascular disease. Plasma samples were analyzed to determine lipid profiles.<sup>22</sup>

For each patient with myocardial infarction (387 men) or angina or revascularization (865 men), a control matched according to age (within one year of the age of the case patient), smoking history (current, past, or none), and time of randomization (in six-month periods) was selected among men eligible on the basis of health status (no reported signs or symptoms of ischemic heart disease). An additional 3 matched controls were identified for 314 case patients with myocardial infarction, and an additional 2 matched controls for the remaining 73 patients with myocardial infarction, to increase the statistical power of the subsample of persons with myocardial infarction.

These procedures yielded 1250 case patients with ischemic heart disease and 2340 controls (2 of the case patients with angina had blood specimens unsuitable for analysis).

#### Determination of ACE Genotypes

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 and size fractionation and visualization by electrophoresis.

One microliter of whole blood was added to 5  $\mu\text{l}$  of GeneReleaser (Bioventures, Murfreesboro, Tenn.) and taken through two cycles of heating to  $97^{\circ}\text{C}$  and cooling to  $8^{\circ}\text{C}$  according to the manufacturer's recommendations. After incubation at  $80^{\circ}\text{C}$  for 30 minutes, 20  $\mu\text{l}$  of a PCR master mix containing 1  $\mu\text{M}$  primers, 200  $\mu\text{M}$  deoxynucleotide triphosphates, 1.3 mM magnesium chloride, 50 mM potassium chloride, 10 mM TRIS-hydrochloric acid (pH 8.4 at  $25^{\circ}\text{C}$ ), 0.1 percent Triton X-100, and 0.35 unit of *Thermus aquaticus* DNA polymerase was added. We used an optimized primer pair to amplify the *D* and *I* alleles, resulting in 319-bp and 597-bp amplicons, respectively (hace3s, 5'GCCCTGCAGGTGTCTGCAGCATGT3'; hace3as, 5'GGATGGCTCTCCCCGCCTTGTCTC3'). The thermocycling procedure (PTC 100 apparatus, MJ Research, Watertown, Mass.) consisted of denaturation at  $94^{\circ}\text{C}$  for 30 seconds, annealing at  $56^{\circ}\text{C}$  for 45 seconds, and extension at  $72^{\circ}\text{C}$  for 2 minutes, repeated for 35 cycles, followed by a final extension at  $72^{\circ}\text{C}$  for 7 minutes. After the addition of 5  $\mu\text{l}$  of a glycerol-based loading buffer, 7  $\mu\text{l}$  of the mixture was loaded onto a 1.5 percent submarine agarose slab (FMC, Rockland, Me.) containing 40 mM TRIS acetate, 2 mM EDTA, and 1  $\mu\text{g}$  of ethidium bromide per milliliter of solution and fractionated according to size at 5 V per centimeter. The amplification products of the *D* and *I* alleles were identified by 300-nm ultraviolet transillumination as distinct bands; in heterozygous samples a third band, assumed to represent a heteroduplex DNA product, was commonly seen (Fig. 1).

Because the *D* allele in heterozygous samples is preferentially amplified,<sup>23</sup> each sample found to have the *DD* genotype was subjected to a second, independent PCR amplification with a primer pair that recognizes an insertion-specific sequence (hace5a, 5'TGGACCA-CAGCGCCCGCCACTAC3'; hace5c, 5'TCGCCAGCCCTCCCAT-GCCCATAA3'), with identical PCR conditions except for an annealing temperature of  $67^{\circ}\text{C}$ . The reaction yields a 335-bp amplicon only in the presence of an *I* allele, and no product in samples homozygous for *DD* (Fig. 1B). This procedure correctly identified the 4 to 5 per-

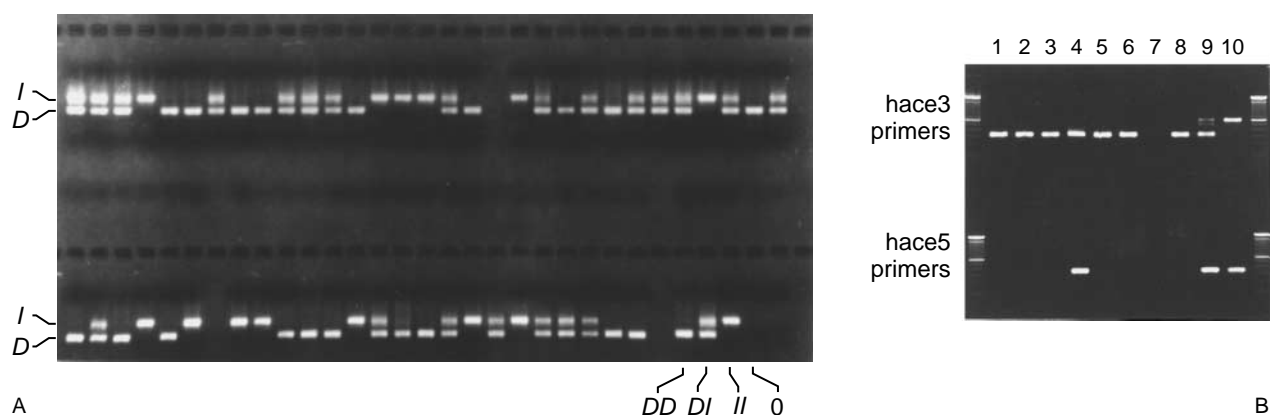


Figure 1. Determination of ACE Genotypes by PCR Amplification.

Samples were loaded into an upper and a lower row of wells in each panel shown. Panel A shows part of a representative 1.5 percent agarose gel stained with ethidium bromide and photographed under ultraviolet transillumination. Ninety samples from case patients and controls were loaded, along with four standards (*DD*, *DI*, *II*, and a reaction with no DNA [0]), which are seen in the lower right-hand corner of the gel.

Panel B shows the method of screening for the erroneous assignment of the *DD* genotype to *DI* samples with use of an insertion-specific primer. At the top, several samples (lanes 1 through 6) are identified as *DD* by the hace3 primer pair, followed by a blank control (lane 7) and three standards for *DD*, *DI*, and *II* (lanes 8 through 10, respectively). The *DI* standard in lane 9 is an example of preferential amplification of the *D* band as compared with the *I* band; a presumed heteroduplex band (faint line below the *I* band) is also present. At the bottom, the same samples are shown amplified with the hace5 primer pair, which recognizes insertion-specific sequences. A sample previously misclassified as *DD* appears in lane 4.

cent of samples with the *DI* genotype that are misclassified as *DD* with the insertion-spanning primers.

The PCR results were scored by two independent investigators who did not know whether the sample was from a case patient or a control. No intraobserver variability was found on repeated readings of the same gel, and the interobserver variability was less than 1 percent. All ambiguous samples were analyzed a second time; a second analysis was necessary for only 27 of the 3590 samples studied.

As an additional measure of quality control, the group of blood samples from case patients and controls received at the genotyping laboratory was randomly and anonymously "spiked" with standards, all of which were interpreted accurately. We also compared data on the genotypes of 30 samples obtained from Dr. François Cambien and found 100 percent concordance between his results and ours.

### Other Laboratory Measurements

Plasma levels of apolipoprotein B had previously been determined in a subgroup of patients with myocardial infarction and in their matched controls. Plasma was obtained by centrifugation of EDTA-anticoagulated blood at 4°C and 2500×g for 20 minutes, and apolipoprotein B levels were measured with a previously described specific radioimmunoassay.<sup>24</sup>

### Statistical Analysis

The frequencies of the alleles and genotypes among the case patients and controls were counted and were compared by the chi-square test with the values predicted by the assumption of Hardy-Weinberg equilibrium in the sample. Odds ratios were calculated as a measure of the association of the *ACE* genotype with the phenotype of ischemic heart disease, with the effects of the *D* allele assumed to be additive (with scores of 0, 1, and 2 assigned for *II*, *DI*, and *DD*, respectively), dominant (with scores of 0 for *II* and 1 for *DI* and *DD* combined), or recessive (with scores of 0 for *II* and *DI* combined and 1 for *DD*). For each odds ratio we calculated two-tailed P values and 95 percent confidence intervals. We performed both matched-pair and unmatched analyses, with adjustment for additional risk factors (exercise, alcohol use, and history of hypertension or hypercholesterolemia) by conditional and unconditional logistic regression, respectively.<sup>25</sup> To duplicate the identification of a "low risk" subgroup in an earlier study,<sup>6</sup> we partitioned our sample at the median value for body-mass index (the weight in kilograms divided by the square of the height in meters), in the case of ischemic heart disease, and the median values for body-mass index and apolipoprotein B, in the myocardial-infarction subgroup, since this seemed to approximate most closely the procedure used previously.

## RESULTS

### Characteristics of the Study Population

The mean ages of the case patients and controls in the study sample were 59.5±8.7 and 59.1±8.6 years, respectively (P not significant), with a mean follow-up period of 9.7±0.9 years. In the overall sample, 11.1 percent of the case patients and 13.4 percent of the controls were current smokers, 46.9 percent of the case patients and 43.8 percent of the controls were former smokers, and 41.9 percent of the case patients and 42.6 percent of the controls had never smoked (P not significant for any comparison). These results reflect the

Table 1. Base-Line Characteristics of the Case Patients with Ischemic Heart Disease and Their Matched Controls.\*

CHARACTERISTIC†	ALL SUBJECTS		SUBGROUP WITH MYOCARDIAL INFARCTION		SUBGROUP WITHOUT MYOCARDIAL INFARCTION	
	CASE PATIENTS	CONTROLS	CASE PATIENTS	CONTROLS	CASE PATIENTS	CONTROLS
No. of patients	1250	2340	387	1475	863	865
Age (yr)	59.5±8.7	59.1±8.6	59.5±8.7	58.9±8.2	59.4±8.7	59.4±8.7
Smoking history						
Never	41.9	42.6	42.8	43.7	41.4	41.6
Former	46.9	43.8	41.4	40.6	49.5	49.4
<1 pack/day	4.0	5.1	5.2	6.3	3.5	3.0
≥1 pack/day	7.2	8.2	10.6	9.4	5.6	6.0
Body-mass index‡	25.3±3.0	25.0±3.0	25.5±3.3	25.0±3.1	25.2±2.9	24.9±2.9
Alcohol use						
Daily	23.4	31.5	22.0	30.3	24.1	33.6
Weekly	47.5	44.4	47.9	44.5	47.3	44.3
Monthly	12.1	9.3	13.2	9.9	11.5	8.1
Rarely or never	17.0	14.8	16.9	15.3	17.1	14.0
History of hypertension	28.1	16.7	29.3	17.0	27.6	16.2
History of hypercholesterolemia	15.3	7.7	16.3	8.4	14.8	6.6
History of diabetes mellitus	6.5	2.4	5.5	2.9	6.5	1.7
Exercise (weekly or more)	67.6	73.3	65.1	73.5	68.8	73.1
Aspirin treatment§	48.1	49.9	39.8	50.2	51.8	49.5
Apolipoprotein B (mg/dl)	—	—	113.9±34.9	109.6±34.9	—	—
Total cholesterol (mg/dl)	—	—	226.0±38.5	213.1±35.9	—	—
HDL cholesterol (mg/dl)¶	—	—	43.8±11.7	48.2±11.5	—	—

\*Plus-minus values are means ±SD; except as stated otherwise, other values are percentages of the group. Lipid profiles were measured only in the myocardial-infarction subgroup.

†Age and smoking history were controlled for by the matched-pair design.

‡Calculated by dividing the weight in kilograms by the square of the height in meters.

§Aspirin was given by random assignment through January 25, 1988.

¶HDL denotes high-density lipoprotein.

matching characteristics (age and smoking) used to select the case patients and controls. The distribution of several other risk factors among the case patients and controls is shown in Table 1 and reflects the expected differences in the prevalence of recognized risk factors.

### Frequencies of Alleles and Genotypes

Among the controls, the *I* and *D* alleles had frequencies of 44.49 and 55.51 percent, respectively. The frequencies of the *DD*, *II*, and *DI* genotypes (30.89, 19.87, and 49.23 percent, respectively) were virtually identical to those predicted by the Hardy-Weinberg equilibrium (30.81, 19.79, and 49.39 percent; chi-square=0.01; P=0.92). The frequencies of alleles determined separately among the controls with and without myocardial infarction did not differ significantly from those in the overall control sample and were, in either case, in agreement with the frequencies predicted by the Hardy-Weinberg equilibrium. The same was true of the overall study sample, in which the observed frequencies of the *I* and *D* alleles were 43.81 and 56.19 percent, respectively.

None of the recognized risk factors shown in Table 1 differed in distribution or mean value according to *ACE* genotype (data not shown).

### Associations between Genotype and Phenotype

Genotype frequencies in the overall sample are shown in Table 2 according to the patient's status as a case patient or a control. The relative risk of ischemic

Table 2. Frequencies of the *ACE* Genotypes in the Case Patients with Ischemic Heart Disease and the Controls.

GROUP AND GENOTYPE	CASE PATIENTS		CONTROLS	
	no. (%)			
Overall group with ischemic heart disease				
<i>DD</i>	409 (32.7)		723 (30.9)	
<i>DI</i>	619 (49.5)		1152 (49.2)	
<i>II</i>	222 (17.8)		465 (19.9)	
All	1250 (100)		2340 (100)	
Subgroup with low risk of ischemic heart disease*				
<i>DD</i>	188 (32.6)		372 (30.6)	
<i>DI</i>	288 (50.0)		603 (49.5)	
<i>II</i>	100 (17.4)		242 (19.9)	
All	506 (100)		1155 (100)	
Subgroup with myocardial infarction				
<i>DD</i>	126 (32.6)		453 (30.7)	
<i>DI</i>	190 (49.1)		725 (49.2)	
<i>II</i>	71 (18.3)		297 (20.1)	
All	387 (100)		1475 (100)	
Subgroup with low risk of myocardial infarction†				
<i>DD</i>	32 (30.5)		214 (31.3)	
<i>DI</i>	57 (54.3)		339 (49.6)	
<i>II</i>	16 (15.2)		131 (19.1)	
All	105 (100)		684 (100)	

\*Subjects in this group each had ischemic heart disease and a body-mass index below the median value (24.5).

†Subjects in this group each had myocardial infarction and a body-mass index and a serum apolipoprotein B concentration below the median (24.7 and 109.1 mg per deciliter, respectively).

heart disease conferred by the *D* allele (assuming an additive effect) was 1.08 in the matched-pair analysis (95 percent confidence interval, 0.90 to 1.20;  $P=0.16$ ) (Table 3); for *DD*, as compared with the *DI* and *II* genotypes (assuming a recessive effect of the *D* allele, as has been done formerly<sup>6</sup>), it was 1.09 (95 percent confidence interval, 0.92 to 1.27;  $P=0.3$ ). These data are unadjusted except for smoking history and age, as specified by the study design. Additional analyses assuming a dominant effect of the *D* allele did not change this result. Likewise, matched-pair analyses (data not shown) and unmatched analyses (Table 4) adjusted for covariates failed to demonstrate an effect of the *ACE* genotype on the phenotype.

Separate subgroup analyses of pairs in which the case patient was defined according to whether he had a myocardial infarction, using the same models and analytic algorithms, showed no effect of either *ACE* allele on the patient's status as a case patient or control (Tables 2 and 3; data not shown for the entire model).

Analysis of a "low risk" subgroup as defined by a body-mass index below the median value (24.5) and of a subgroup from the myocardial-infarction sample with body-mass index and apolipoprotein B values below the medians (24.7 and 109.1 mg per deciliter, respectively) demonstrated again that there was no association between *ACE* genotype and disease status (Tables 2 and 3).

## DISCUSSION

Heritable factors, in combination with a number of recognized environmental risk factors, are important

determinants of the pathogenesis and natural history of ischemic heart disease. The notion that the presence of the *D* allele may identify *ACE* as one of the genes contributing to an increased risk of ischemic heart disease is both intriguing and provocative. The range of hemodynamic effects of an activated renin-angiotensin system, in the context of a correlation between the *ACE* genotype and plasma ACE activity, is compatible with the concept of an increased risk of myocardial ischemia conferred by the *DD* genotype. However, our results in a large, prospective study do not support the postulated role of the *ACE* genotype as a marker for either myocardial infarction or ischemic heart disease. In fact, the upper bounds of the 95 percent confidence intervals were 1.24 for ischemic heart disease and 1.25 for myocardial infarction, rendering the relative risk of 1.34 reported in retrospective data<sup>6</sup> very unlikely in the sample we studied.

How can we explain the discrepancy in findings between the present investigation and the earlier study?<sup>6</sup> The most likely reasons are differences in the criteria used to select the patients and controls and the possibility of differences in genetic background in the samples examined, confounded by the low informativeness of the marker used.

The differences in patient selection between the two studies are highlighted by the fact that patients were recruited into the earlier study from three to nine months after their myocardial infarctions<sup>6</sup>; thus, none of those who died within this period were represented. In contrast, our study design included all myocardial infarctions, even if they were associated with early death, if a proper diagnosis could be established. The generalizability of our findings is limited by the low risk profile overall and the low incidence of ischemic

Table 3. Analysis of the Risk of Ischemic Heart Disease According to Genotype in the Overall Sample and Selected Subgroups.

GROUP AND MODEL STUDIED	ODDS RATIO (95% CI)*	P VALUE
Overall group with ischemic heart disease		
<i>DD</i> vs. <i>DI</i> vs. <i>II</i> †	1.08 (0.92–1.20)	0.16
<i>DD</i> vs. <i>DI</i> and <i>II</i> ‡	1.09 (0.92–1.27)	0.30
<i>DD</i> and <i>DI</i> vs. <i>II</i> §	1.13 (0.94–1.36)	0.20
Subgroup with low risk of ischemic heart disease¶		
<i>DD</i> vs. <i>DI</i> vs. <i>II</i> †	1.14 (0.95–1.38)	0.17
<i>DD</i> vs. <i>DI</i> and <i>II</i> ‡	1.10 (0.89–1.36)	0.37
<i>DD</i> and <i>DI</i> vs. <i>II</i> §	1.18 (0.91–1.53)	0.21
Subgroup with myocardial infarction		
<i>DD</i> vs. <i>DI</i> vs. <i>II</i> †	1.08 (0.92–1.26)	0.38
<i>DD</i> vs. <i>DI</i> and <i>II</i> ‡	1.09 (0.85–1.38)	0.50
<i>DD</i> and <i>DI</i> vs. <i>II</i> §	1.12 (0.84–1.49)	0.44
Subgroup with low risk of myocardial infarction		
<i>DD</i> vs. <i>DI</i> vs. <i>II</i> †	0.95 (0.67–1.35)	0.78
<i>DD</i> vs. <i>DI</i> and <i>II</i> ‡	0.96 (0.62–1.51)	0.87
<i>DD</i> and <i>DI</i> vs. <i>II</i> §	1.32 (0.75–2.33)	0.34

\*CI denotes confidence interval.

†Additive effect of the *D* allele.

‡Recessive effect of the *D* allele.

§Dominant effect of the *D* allele.

¶Subjects in this group each had ischemic heart disease and a body-mass index below the median value (24.5).

||Subjects in this group each had myocardial infarction and a body-mass index and serum apolipoprotein B concentration below the median (24.7 and 109.1 mg per deciliter, respectively).

Table 4. Odds Ratios for Ischemic Heart Disease According to *ACE* Genotype and Other Variables, by Multivariate Analysis.\*

VARIABLE	ISCHEMIC HEART DISEASE		SUBGROUP WITH MYOCARDIAL INFARCTION		SUBGROUP WITHOUT MYOCARDIAL INFARCTION	
	ODDS RATIO (95% CI)	P VALUE	ODDS RATIO (95% CI)	P VALUE	ODDS RATIO (95% CI)	P VALUE
<i>DD</i> vs. <i>DI</i> vs. <i>II</i> †	1.07 (0.96–1.19)	0.24	1.05 (0.89–1.25)	0.56	1.05 (0.90–1.22)	0.52
<i>DD</i> vs. <i>DI</i> and <i>II</i> ‡	1.04 (0.89–1.22)	0.61	1.04 (0.80–1.36)	0.75	1.00 (0.81–1.26)	0.94
<i>DD</i> and <i>DI</i> vs. <i>II</i> §	1.16 (0.95–1.40)	0.14	1.11 (0.81–1.51)	0.51	1.16 (0.88–1.51)	0.29
Alcohol use						
Daily	0.72 (0.56–0.91)	0.007	0.65 (0.43–0.97)	0.03	0.71 (0.51–0.99)	0.04
Weekly	1.00 (0.80–1.24)	0.99	0.96 (0.68–1.37)	0.81	1.03 (0.76–1.40)	0.86
Monthly	1.18 (0.88–1.59)	0.26	1.07 (0.67–1.69)	0.79	1.49 (0.97–2.29)	0.07
History of hypertension	1.92 (1.61–2.30)	<0.001	2.06 (1.55–2.74)	<0.001	1.97 (1.52–2.55)	<0.001
History of hypercholesterolemia	2.04 (1.61–2.58)	<0.001	1.99 (1.39–2.84)	<0.001	2.54 (1.77–3.66)	<0.001
History of diabetes mellitus	2.36 (1.62–3.41)	<0.001	1.98 (1.13–3.46)	0.017	3.05 (1.68–5.56)	<0.001
Exercise (weekly or more)	0.78 (0.66–0.92)	<0.003	0.69 (0.53–0.90)	0.006	0.84 (0.67–1.06)	0.15
Aspirin treatment	0.92 (0.80–1.07)	0.30	0.63 (0.49–0.81)	<0.001	1.14 (0.92–1.40)	0.23

\*No data are given for age or smoking history because these variables were controlled for by the matched-pairs design. CI denotes confidence interval.

†Additive effect of the *D* allele.

‡Recessive effect of the *D* allele.

§Dominant effect of the *D* allele.

heart disease among physicians; given the exaggerated effect of the *ACE* polymorphism previously reported in low-risk populations, an opposite bias should have been introduced. Whereas the case patients in our study were an average of 5.5 years older than those in the earlier study, this difference does not provide a likely explanation for the discrepancy, because two recent studies in small cohorts<sup>26,27</sup> indicated that an increased risk was also associated with the *DD* genotype in older subjects.

In discussing the possible role of the *ACE* genotype as a risk factor for the occurrence of disease, one must bear in mind that although it is not impossible, it is rather unlikely that the intronic *D–I* polymorphism in itself is a disease-causing mutation. It may, however, exist in linkage disequilibrium with a putative pathogenetic mutation elsewhere in the *ACE* gene and therefore serve as a useful genetic marker. Among unrelated people, such linkage disequilibrium is maintained only if the marker and the disease mutation are in very close proximity on the chromosome, and the disequilibrium depends on their relative allelic frequencies. Highly informative markers are usually present in a large number of allelic variants, with individual alleles with low frequencies conferring high specificity. At the other end of the spectrum, the specificity of a diallelic marker system with similar allelic frequencies, exemplified by the *ACE D–I* polymorphism, can be expected to be low, but the polymorphism may still be useful in a genetic isolate in which both the marker and disease mutations are of similar phylogenetic antiquity. Admixture from populations in which no such linkage disequilibrium prevails will rapidly interfere with the usefulness of the marker.

It is possible that the presumably more heterogeneous genetic background of a North American population, as compared with somewhat less ethnically diverse European cohorts, may account for a decline in the degree of linkage disequilibrium between the *D–I*

marker and a putative disease mutation, thus yielding negative results. Therefore, our study does not rule out the possibility that certain mutant alleles of the *ACE* gene may be associated with a predisposition to ischemic heart disease; it simply indicates that in middle-aged U.S. men the *ACE D–I* polymorphism does not serve as a useful indicator of a putative disease-causing mutation on the *ACE* gene.

Recently, a number of small case-control studies have reported an association between the prevalence of the *ACE* genotype and such diverse entities as dilated cardiomyopathy,<sup>28</sup> coronary-artery restenosis,<sup>29</sup> hypertrophic cardiomyopathy,<sup>30</sup> parental history of myocardial infarction,<sup>31,32</sup> and cardiac hypertrophy,<sup>33</sup> with variable results.<sup>34,35</sup> It is important to recognize that studies of linkage disequilibrium (association) are highly sensitive to the selection of a genetically appropriate control sample. Indeed, the frequency of the *DD* genotype in the control samples in these studies ranged from 17 percent<sup>33</sup> to 39 percent,<sup>34</sup> indicating major inconsistencies in the selection of controls. The frequency of the *DD* genotype in our controls was 31 percent, a figure that is in agreement with the originally published data<sup>9,10,18</sup> and was independently verified by the genotyping of an additional, large cross-population sample consisting of more than 3900 people (unpublished data), providing a sample for our estimates of *D* and *I* allelic frequencies in normal populations that exceeds those of previous analyses. It should be emphasized that the frequency of the *DD* genotype reported here among the case patients (but also among the controls) is identical to that found among the case patients in the original study.<sup>6</sup> It is interesting to note, in hindsight, that our earliest preliminary results were flawed by a chance underrepresentation of the *DD* genotype in a small cohort of some 400 controls; this problem was corrected once the sample was expanded.<sup>36</sup>

Primarily on the basis of the successful reduction of morbidity and mortality after myocardial infarction by

treatment with ACE inhibitors,<sup>37-39</sup> we believe that the renin-angiotensin system is likely to play an important part in ischemic heart disease. However, the mechanism has so far remained elusive. Although the present data do not exclude a pathogenetic role of mutations of the *ACE* gene in ischemic heart disease and myocardial infarction, the results of this large, prospective investigation indicate that the *ACE D-I* polymorphism is not useful for assessing the risk of ischemic heart disease or myocardial infarction.

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