Angiotensin I-converting enzyme gene polymorphism, coronary artery disease and myocardial infarction

An angiographically controlled study

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Objectives We investigated the association between insertion/deletion polymorphism of the angiotensin I-converting enzyme (ACE) gene, the presence and extent of coronary artery disease, and myocardial infarction.

Background The D allele of the ACE gene has been associated with coronary artery disease and myocardial infarction, but this association has been challenged in epidemiological studies.

Methods Nine hundred and sixty-nine men and 341 women undergoing coronary angiography were studied. The ACE genotypes were assessed by polymerase chain reaction from genomic deoxyribonucleic acid, homozygosity for the D allele was controlled using an insertion-specific primer. Coronary artery disease was defined by angiographic criteria, the extent of coronary artery disease by the number of coronary arteries with ≥50% lumen narrowing.

Results The ACE genotypes did not differ in terms of age, sex, body mass index, blood pressure, plasma lipids or lipoproteins. We found no association between the ACE genotypes and coronary artery disease (odds ratio, 95% confidence interval in DD genotypes for coronary artery disease in men 0·97, 0·70–1·36; in women 1·56, 0·95–2·57), extent of coronary artery disease (men 1·17, 0·85–1·61; women 1·24, 0·65–2·34), or myocardial infarction among the patients with coronary artery disease (men 1·07, 0·78–1·48; women 0·95, 0·50–1·76). The ACE genotype was not associated with coronary artery disease or myocardial infarction in hypertensives (n=771; odds ratio for coronary artery disease 0·93, 0·65–1·34; odds ratio for myocardial infarction 0·94, 0·66–1·33), or in patients ≤60 years (n=649; odds ratio for coronary artery disease 1·05, 0·72–1·52; odds ratio for myocardial infarction 0·96, 0·63–1·47).

Conclusion ACE insertion/deletion gene polymorphism is associated neither with the prevalence nor the extent of coronary artery disease, nor with myocardial infarction in this relatively large sample of Caucasian men and women. Genotyping for ACE insertion/deletion polymorphism is not useful in assessing the individual risk of coronary artery disease or myocardial infarction.

Key Words: ACE gene polymorphism, coronary heart disease, myocardial infarction.
development of myocardial infarction or other manifestations of coronary artery disease\textsuperscript{[10]}. A recent study in patients undergoing coronary angiography implied that ACE insertion/deletion polymorphism is not associated with coronary lesions themselves, but with the occurrence of myocardial infarction in patients with coronary heart disease\textsuperscript{[11]}. Similar results were obtained in an Australian population, in whom there was a strong association of the ACE DD genotype with the presence of coronary artery disease or a history of myocardial infarction, but not with the severity of coronary artery disease, in comparison to a cohort of healthy schoolchildren\textsuperscript{[12]}.

There are several possible reasons for these discrepancies. Besides the different genetic backgrounds of the study populations, some of the studies cited have only minor statistical power, or the associations between ACE gene polymorphism and coronary artery disease or myocardial infarction have been restricted to relatively small subgroups. In some of the studies the presence or absence of coronary artery disease was not determined by angiography, and, as a consequence, might have used false-negative control populations. A recent meta-analysis comprising 8873 subjects from 15 studies found an association between the ACE D allele and myocardial infarction, but there may have been a bias in the smaller studies towards positive results\textsuperscript{[13]}. Thus, the purpose of this study was first to determine the relationship between ACE insertion/deletion polymorphism and the presence or extent of coronary artery disease, and second the association with myocardial infarction among patients with angiographically documented coronary artery disease in a large cohort of middle-European men and women undergoing cardiac catheterization.

**Patients and methods**

**Patients**

Our study population consisted of 1310 patients (969 male, 341 female) who had undergone coronary angiography in the University Hospital of Tübingen, Germany, because of symptoms possibly related to coronary artery disease. Relevant history, cardiovascular risk factors, and current treatment were obtained from each patient using a standard questionnaire, and the data were validated with reference to hospital case records. The history of myocardial infarction was confirmed according to standard criteria, i.e. two or more of the following: history of chest pain indicative of myocardial infarction, increase in creatine kinase and creatine kinase MB levels > threefold the upper reference limit during follow-up, and characteristic electrocardiographic changes at the time of diagnosis (ST-segment elevation >0·1 mV in at least two leads). Smokers were defined as current smokers and patients who had ceased smoking; non-smokers were defined as patients with no history of prior or current smoking. Diabetes mellitus was recorded according to WHO criteria\textsuperscript{[14]}. Hypertension was defined as a blood pressure of 160/95 mmHg or greater on repeated measurements and/or current use of antihypertensive drugs due to a previous history of arterial hypertension. Hyperlipidaemia was defined as plasma total cholesterol above 6·5 mmol·l\textsuperscript{-1} and/or plasma triglycerides above 2·0 mmol·l\textsuperscript{-1} and/or current use of lipid-lowering drugs with an established diagnosis of hyperlipidaemia. Since the ACE DD genotype has been postulated to be associated with ischaemic and idiopathic dilated cardiomyopathy\textsuperscript{[15]}, patients with cardiomyopathy were not included in this study. The study was approved by the local Ethical Committee, and informed consent was obtained from each patient before the procedure.

**Coronary angiography**

All patients underwent diagnostic coronary angiography by the Judkins technique\textsuperscript{[16]}. The angiograms were assessed by two independent cardiologists. Each angiogram was classified as revealing either no coronary lesion with a luminal obstruction of at least 50\%, or one, two, or three major epicardial coronary arteries causing a luminal stenosis equal to or more than 50\%.

**Laboratory methods**

To analyse the lipid profile and to extract genomic DNA peripheral venous blood samples were drawn from all patients after overnight fasting. Plasma total cholesterol and triglycerides were measured by enzymatic methods (Boehringer Mannheim, Germany) after overnight fasting, HDL-cholesterol was determined after sodium phosphotungstate/magnesium chloride precipitation\textsuperscript{[17]}. In all patients with plasma triglycerides \(<400\text{ mg} \cdot \text{dl}^{-1}\), LDL-cholesterol was calculated according to Friedewald’s formula\textsuperscript{[18]}.

Genomic DNA was isolated and purified from whole blood (EDTA) using QIAamp-spin-columns, according to the protocol given by the manufacturer (QIAamp Blood Kit, QIAGEN GmbH, Hilden, Germany). The ACE genotypes were assessed by polymerase chain reaction using primer sequences and polymerase chain reaction cycling conditions, as described previously\textsuperscript{[19]}. The polymerase chain reaction fragments were separated on a horizontal 5\% polyacrylamide gel by electrophoresis and visualized by staining with silver solution. All gels were read by two independent observers (M.P and S.P.). According to the absence or presence of the 287 base pair insertion in the polymerase chain reaction product, the patients were classified as homozygous DD or II, or heterozygous ID. To prevent mistyping of ID as DD genotypes, a second polymerase chain reaction with an insertion specific primer (5’TTTGAGACGGAGTCTCGCTC3’) was performed in all samples classified as

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Table 1 Characteristics of the patients without and with coronary artery disease, and with a history of myocardial infarction

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th></th>
<th>Men</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without CAD</td>
<td>With CAD</td>
<td>With MI</td>
<td>Without CAD</td>
<td>With CAD</td>
<td>With MI</td>
</tr>
<tr>
<td>n</td>
<td>142</td>
<td>199</td>
<td>79</td>
<td>203</td>
<td>766</td>
<td>432</td>
</tr>
<tr>
<td>Age (years)</td>
<td>59.2 ± 0.8</td>
<td>64.8 ± 0.7***</td>
<td>62.9 ± 1.0*</td>
<td>56.9 ± 0.7</td>
<td>60.4 ± 0.3***</td>
<td>59.9 ± 0.4</td>
</tr>
<tr>
<td>Body mass index (kg · m⁻²)</td>
<td>26.1 ± 0.5</td>
<td>27.6 ± 0.4*</td>
<td>28.3 ± 0.7</td>
<td>26.9 ± 0.3</td>
<td>27.0 ± 0.1*</td>
<td>26.9 ± 0.2</td>
</tr>
<tr>
<td>Smoker (current or ex-smoker, %)</td>
<td>11.5%</td>
<td>29.6%***</td>
<td>36.2%</td>
<td>59.9%</td>
<td>69.8%*</td>
<td>70.0%</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>14.0%</td>
<td>31.7%***</td>
<td>34.9%</td>
<td>16.4%</td>
<td>21.3%</td>
<td>22.8%</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>145.2 ± 2.5</td>
<td>150.5 ± 2.3</td>
<td>146.5 ± 3.5</td>
<td>129.6 ± 2.1</td>
<td>134.9 ± 1.1*</td>
<td>131.6 ± 1.5</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>79.2 ± 1.1</td>
<td>76.7 ± 1.0</td>
<td>75.3 ± 1.6</td>
<td>79.8 ± 1.0</td>
<td>77.8 ± 0.5</td>
<td>77.3 ± 0.7</td>
</tr>
<tr>
<td>Total cholesterol (mmol · l⁻¹)</td>
<td>4.90 ± 0.09</td>
<td>5.26 ± 0.08**</td>
<td>5.20 ± 0.12</td>
<td>4.60 ± 0.07</td>
<td>4.84 ± 0.04**</td>
<td>4.89 ± 0.05</td>
</tr>
<tr>
<td>LDL cholesterol (mmol · l⁻¹)</td>
<td>2.91 ± 0.08</td>
<td>3.19 ± 0.07**</td>
<td>3.15 ± 0.11</td>
<td>2.72 ± 0.05</td>
<td>2.97 ± 0.03***</td>
<td>2.97 ± 0.04</td>
</tr>
<tr>
<td>HDL cholesterol (mmol · l⁻¹)</td>
<td>1.21 ± 0.03</td>
<td>1.11 ± 0.03**</td>
<td>1.06 ± 0.04</td>
<td>1.00 ± 0.02</td>
<td>0.94 ± 0.01*</td>
<td>0.95 ± 0.01</td>
</tr>
<tr>
<td>Triglycerides (mmol · l⁻¹)</td>
<td>1.80 ± 0.09</td>
<td>2.15 ± 0.08***</td>
<td>2.20 ± 0.13</td>
<td>1.95 ± 0.08</td>
<td>2.11 ± 0.04*</td>
<td>2.17 ± 0.05</td>
</tr>
<tr>
<td>Lipoprotein (a) (mg · dl⁻¹)</td>
<td>30.5 ± 2.9</td>
<td>40.7 ± 2.4***</td>
<td>37.7 ± 4.1</td>
<td>26.7 ± 2.3</td>
<td>56.9 ± 1.2***</td>
<td>37.3 ± 1.6</td>
</tr>
</tbody>
</table>

Data are n, means ± SE, or %; CAD=coronary artery disease; MI=myocardial infarction. Statistical significance *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.

The characteristics of the 1310 study patients are shown in Table 1. In both women and men, the significantly different results (age, smoking status, total, LDL, and HDL cholesterol, triglycerides, and lipoprotein (a)) between patients with established or excluded coronary artery disease are well in agreement with established cardiovascular risk factors.

Genotype distribution

The distribution of the ACE genotypes in the study population was in Hardy-Weinberg equilibrium (Table 2). The genotype groups were well matched for age, body mass index, smoker status, and prevalence of diabetes mellitus. Notably, systolic and diastolic blood pressure were not different between the groups, and there were also no differences in the plasma lipids and lipoproteins. The allele frequencies in both women (D 0.537, I 0.463) and men (D 0.546, I 0.454) were comparable to those obtained in populations from Europe[2,8,10], and North America[9].

ACE genotype and coronary artery disease

We found no significant association between ACE insertion/deletion polymorphism and coronary artery disease, comparing the patients with at least one significantly diseased vessel with the patients with angiographically excluded coronary artery stenosis (Table 3). We found also no association between the ACE genotype and the extent of coronary artery disease. In the patients with one, two, or three significantly diseased coronary arteries the frequencies of the D allele were 0.591, 0.491, and 0.563 among the females, and 0.561, 0.491, and 0.563 among the males.

Results

The results for continuous variables are expressed as means ± standard deviations. The means of the three genotype groups (DD, ID, II) were compared in a one-way analysis of variance (plasma triglycerides and lipoprotein (a) after log transformation). We determined whether the distribution of the ACE genotypes was in Hardy-Weinberg equilibrium using chi-squared analysis, as described by Emery[21]. Categoric data and the frequencies of the alleles and genotypes were assessed by the likelihood ratio test. For the ACE genotype, odds ratios were calculated as a measure of the association with the presence or absence of coronary artery disease, the extent of coronary artery disease, and myocardial infarction. The effects of the D allele were either assumed to be recessive (with scores of 0 for the II and ID genotypes, and 1 for the DD genotypes), or dominant (with scores of 0 for the II genotypes, and 1 for the ID and DD genotypes). For each odds ratio, the 95% confidence intervals were calculated. The analysis was also carried out by means of an explorative multiple logistic regression analysis to assess the independent role of the different factors possibly influencing the presence or absence of coronary artery disease, the extent of coronary artery disease, and myocardial infarction (dependent variables), and age, sex, smoking, body mass index, diabetes, hypertension, the plasma lipids and lipoproteins, and the ACE genotype as independent variables. All analyses were done using a personal computer with JMP 3.2 software (SAS Institute, Cary, North Carolina). A P value of less than 0.05 was considered to be statistically significant.
The unadjusted odds ratio for having coronary artery disease conferred by the D allele in women was 1.56 (95% confidence interval (CI), 0.95–2.57) assuming it was recessive and 0.95 (95% CI, 0.55–1.61) assuming it was dominant. In men, the odds ratios were 0.97 (95% CI, 0.70–1.36) for recessive and 1.23 (95% CI 0.95–1.76) for dominant effects (Table 3).

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**ACE genotype and myocardial infarction**

Among the 965 patients with angiographically documented coronary artery disease, a positive history of myocardial infarction was found in 511 patients and could be excluded in 418 patients. Thirty-six patients, in whom the history of myocardial infarction could not be excluded or confirmed according to the criteria mentioned above, were excluded from this analysis. In the remaining 929 patients, we found no significant association between ACE insertion/deletion polymorphism and previous myocardial infarction. There was a positive history of myocardial infarction in 55.7% of the DD genotypes (men 59.6%, women 40.7%), in 54.0% of the ID genotypes (men 56.9%, women 42.9%), and in 56.3% of the II genotypes (men 60.4%, women 40.0%) (chi-squared=0.36; P=0.84 for all patients, chi-squared=0.74; P=0.69 for men; chi-squared=0.12; P=0.94 for women). The unadjusted odds ratios for myocardial infarction were 0.94 (95% CI, 0.68–1.28) assuming a dominant effect of the D allele, and 1.04 (95% CI, 0.79–1.38) for the recessive effect of the D allele.

**ACE genotype, coronary artery disease, and myocardial infarction in the subgroup ≤60 years**

Since the effects of the ACE genotype on coronary artery disease or myocardial infarction could be masked by environmental factors in elderly patients, we tested the hypothesis that the ACE genotype confers a risk of coronary artery disease or myocardial infarction in younger subjects by carrying out a subgroup analysis in the patients ≤60 years. In this subgroup (n=694) the ACE genotype was neither associated with the presence of coronary artery disease (odds ratio for the dominant effect of the D allele 1.32 (95% CI 0.88–1.97), the recessive effect 1.05 (95% CI 0.72–1.53)) nor with the number of diseased coronary arteries (odds ratio for three-vessel disease: dominant effect 1.12 (95% CI 0.66–1.96); recessive effect 1.04 (95% CI 0.66–1.64)). In addition, among the patients ≤60 years old with angiographically documented coronary artery disease (n=449) there was also no association between the ACE genotype and the history of myocardial infarction (odds ratio for the dominant effect of the D allele 1.01 (95% CI 0.62–1.61); recessive effect 0.96 (95% CI 0.63–1.47)).

**ACE genotype, coronary artery disease, and myocardial infarction in the hypertensive subgroup**

To test the hypothesis that the deletion allele of the ACE gene confers a risk of coronary artery disease or myocardial infarction in patients with impaired blood pressure regulation, a further subgroup analysis was carried out in the hypertensive patients. Hypertension was defined as blood pressure ≥160/95 mmHg and/or current medical antihypertensive therapy due to an established diagnosis of arterial hypertension. According to this definition, there were 771 patients with hypertension, with a prevalence of coronary artery disease in 77.2%. Again, we found no association between the ACE I/D genotypes and the presence of coronary artery disease. The relative risk for coronary artery disease conferred by the D allele was 1.02 (95% CI 0.67–1.52) for the dominant and 0.93 (95% CI 0.65–1.34) for the recessive effect. There was also no significant association with the extent of coronary artery disease (odds ratio for the dominant effect of the D allele 1.01 (95% CI 0.67–1.53); recessive effect 1.14 (95% CI 0.79–1.64)), or with the history of myocardial infarction (odds ratio for the dominant effect 0.90 (95% CI 0.61–1.35); recessive effect 0.94 (95% CI 0.66–1.33)).

**Relative role of cardiovascular risk factors**

Because we were not able to find any significant association between ACE I/D gene polymorphism and coronary artery disease or myocardial infarction, we analysed whether the established cardiovascular risk factors in this study population were found by multiple logistic regression analysis (Table 5). As expected, this analysis revealed significant effects on the presence of coronary artery disease of advanced age, elevated LDL cholesterol, positive smoking history, male sex, high lipoprotein (a), diabetes, and hypertension, and a protective effect of high HDL cholesterol. Again, the ACE DD genotype was not associated with coronary artery disease. The only risk factors clearly associated with three-vessel disease were diabetes and advanced age, and male sex was the only risk factor for myocardial infarction among the patients with coronary artery disease.

**Discussion**

There is ample evidence that, besides environmental risk factors, genetic factors contribute to the pathogenesis of coronary artery disease. The deletion polymorphism of one of the candidate genes, the ACE gene, has been shown to be associated with both coronary artery disease and myocardial infarction in several studies, but this association was neither supported by the large prospective study in male North American physicians nor by the recently published comparison between Danish coronary artery disease patients and the participants of the Copenhagen City Heart Study. By evaluating the distribution of ACE I/D gene polymorphism in a large series of patients undergoing coronary angiography, the present study has shown that the D
allele of the ACE gene is neither associated with the presence or extent of coronary artery disease nor with previous myocardial infarction. In addition, the subgroup analysis in hypertensive patients, who might possibly be more susceptible to the consequences of the increased plasma ACE activity caused by the D allele, is also not compatible with the concept of an increased risk of coronary artery disease or myocardial infarction conferred by the DD genotype.

ACE polymorphism and coronary artery disease

In this relatively large group of patients undergoing coronary angiography, no association between the ACE genotype and the presence or extent of coronary artery disease was detected. In comparison with other studies using angiography as a gold standard for the detection of coronary artery disease, our results are well in concordance with the findings of Lindpaintner and colleagues in the American physician study,[9] and are well in agreement with the findings of Ludwig et al.[11]. Gardemann et al.[22], and Arca et al.[23], but in contrast to the associations described in Italian, Japanese, and Australian populations.[3,4,12] The only study showing a relationship between the ACE genotype and extent of coronary artery disease was in a relatively small group of Japanese patients.[24]. In a recent study, an association between the ACE D allele and the extent of coronary artery disease has been limited to the subgroup of younger subjects, while an association with myocardial infarction has been restricted to older subjects.[25]. None of the other angiographically controlled studies revealed an association between the ACE genotype and extent of coronary artery disease.[11,12,22]. The additional subgroup analysis in patients ≤ 60 years of age also did not show a significant association between the ACE genotype and the presence or extent of coronary artery disease. This finding means it is unlikely that the effects of the ACE genotype are masked in older patients by environmental factors. The ACE DD genotype has also been reported as associated with an increased risk of cerebrovascular disease in both patients with hypertension[26] and their parents.[27]. However, in the subgroup with hypertension there was neither a relationship between ACE gene polymorphism and coronary artery disease itself or the extent of coronary artery disease, nor with the history of myocardial infarction.

ACE polymorphism and myocardial infarction

Among the 931 patients with angiographically documented coronary artery disease and a clearly documented or excluded history of myocardial infarction, we found no association between the ACE genotype and myocardial infarction. Our results strongly support the findings of Lindpaintner and colleagues in the American physician study,[9] and are well in agreement with the results of Agerholm-Larsen et al. in the Danish population.[10]. The association between the ACE DD genotype and myocardial infarction, which was first described by Cambien and colleagues in the European subjects of the ECTIM study,[2] has been confirmed in several populations of North American[11], Japanese[4,23,28,29], Italian[3], and Australian[12] descent. However, several different studies did not show this association in populations from Austria[8], Finland[30], North America[9], Denmark[10], Japan[23], and New Zealand[31], and a recent meta-analysis has indicated that besides ethnic differences and possible selection bias in most of these studies, a degree of bias towards

Table 5  Odds ratios for presence of coronary artery disease, three-vessel disease, and myocardial infarction according to ACE genotype and other variables by multiple logistic regression analysis

<table>
<thead>
<tr>
<th>Factor</th>
<th>Presence of CAD</th>
<th>3-vessel disease</th>
<th>History of MI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95%-CI</td>
<td>OR</td>
</tr>
<tr>
<td>Age</td>
<td>3.95</td>
<td>2.40–6.58</td>
<td>1.95</td>
</tr>
<tr>
<td>ACE DD genotype</td>
<td>1.14</td>
<td>0.77–1.70</td>
<td>1.21</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.15</td>
<td>1.42–3.26</td>
<td>1.25</td>
</tr>
<tr>
<td>History of smoking</td>
<td>2.31</td>
<td>1.56–3.42</td>
<td>1.27</td>
</tr>
<tr>
<td>Body mass index</td>
<td>1.32</td>
<td>0.80–2.17</td>
<td>1.14</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.80</td>
<td>1.12–2.98</td>
<td>2.30</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.73</td>
<td>1.17–2.54</td>
<td>1.14</td>
</tr>
<tr>
<td>Total-C</td>
<td>1.03</td>
<td>0.34–3.10</td>
<td>0.75</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.52</td>
<td>0.28–0.95</td>
<td>0.72</td>
</tr>
<tr>
<td>LDL-C</td>
<td>3.00</td>
<td>1.12–8.07</td>
<td>1.11</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.67</td>
<td>0.87–3.19</td>
<td>1.49</td>
</tr>
<tr>
<td>Lipoprotein (a)</td>
<td>1.98</td>
<td>1.21–3.26</td>
<td>1.95</td>
</tr>
</tbody>
</table>

CAD = coronary artery disease; MI = myocardial infarction; OR = odds ratio; CI = confidence interval; D = deletion; BMI = body mass index; LDL = low-density lipoprotein; HDL = high-density lipoprotein; C = cholesterol. Age, body mass index, total C, HDL-C, and LDL-C, triglycerides, lipoprotein (a) were each grouped in quartiles. Hypertension was defined as systolic blood pressure ≥ 160 mmHg at repeated measurements, or the current use of antihypertensive agents due to the confirmed diagnosis of arterial hypertension.

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positive results, at least in the smaller studies, seems likely. In addition, the described association between the ACE DD genotype and myocardial infarction has been limited to even smaller subgroups in some studies, partly without significant associations in the whole sample population.

**Limitations of the study**

Since our study failed to reject the null hypothesis, assessment of the statistical power is essential. The study had an 80% power with alpha < 0.05 to detect a 10% difference in the allele frequencies between cases and controls for the presence or absence of coronary artery disease, and for myocardial infarction history. As in all association studies, the results of this study are sensitive to the composition of the control groups. Since the patients with excluded coronary artery disease had been scheduled to coronary angiography, they do not represent a true control group. However, the multiple logistic regression analysis revealed the influence of essentially all established cardiovascular risk factors on the presence or absence of coronary artery disease. In addition, every other control group not undergoing coronary angiography would introduce the problem of possible false-negative classification. In a control population with a mean age of about 60 years, but without clinical signs of coronary artery disease, a considerable prevalence of coronary artery disease would be likely. This cannot reliably be excluded by the use of non-invasive testing, because, especially in unselected populations, the correct classification rates of the available non-invasive procedures are low. In addition, the genotype frequencies of the patients without coronary artery disease were comparable to the large control groups in the American Physicians study and in the Copenhagen City Heart Study, which makes selection bias less probable.

**Conclusions**

The renin–angiotensin system is highly regulated and may contribute to the development of coronary artery disease and myocardial infarction by several mechanisms. The fact that ACE insertion/deletion gene polymorphism is not associated with coronary artery disease and myocardial infarction in large patient populations does not exclude interactions of this polymorphism with other gene polymorphisms in the renin–angiotensin system, which have been shown to increase the risk of coronary artery disease and myocardial infarction. Furthermore, interactions with coronary artery calcification, patency of infarct-related coronary arteries, post-infarction ventricular dilatation, and accelerated coronary sclerosis after cardiac transplantation have recently been described, although in studies with relatively small patient numbers. However, given the wide distribution of the ACE D allele in all populations described, and the lack of association with the presence or extent of coronary artery disease or with myocardial infarction in this study, we conclude that the ACE genotype cannot be regarded as an useful marker of individual cardiovascular risk, or as a marker associated with an increased risk of myocardial infarction in patients with established coronary artery disease.

**References**


