Angiotensin-Converting Enzyme Insertion/Deletion Gene Polymorphic Variant as a Marker of Coronary Artery Disease

A Meta-analysis

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Background: Many studies have investigated the association between the angiotensin-converting enzyme (ACE) gene insertion (I)/deletion (D) polymorphic variant and coronary artery disease (CAD). However, the evidence is inadequate to draw robust conclusions because most studies were generally small and conducted in heterogeneous samples. To shed light on these inconclusive findings, we conducted a meta-analysis of studies relating the ACE I/D polymorphic variant to the risk of CAD.

Methods: We searched the PubMed database for English-language articles on CAD in humans. We estimated summary odds ratios and explored potential sources of heterogeneity and bias.

Results: The 118 studies chosen for the analysis involved 43,733 cases with CAD and 82,606 controls. The heterogeneity between studies was significant. When we compared homozygotes with the D and I alterations, the ACE I/D polymorphic variant was associated with a 25% increased risk of CAD (odds ratio, 1.25; 95% confidence interval, 1.16-1.35). Subgroup analyses for myocardial infarction, diabetes mellitus, male sex, white race, East Asian subjects, and Turkish subjects showed significant associations. No association was found in other racial/ethnic groups, in women, in premature cases, or in cases with low levels of risk factors. The major sources of heterogeneity were due to racial/ethnic diversity, genotyping procedure, and age matching. Cumulative meta-analysis for the allelic contrast showed a trend of association as information accumulated. There was a differential magnitude of effect in large vs small studies.

Conclusions: The meta-analysis demonstrated evidence of a modest positive association between ACE I/D polymorphic variant and CAD. The meta-analysis also highlights the heterogeneity of reported results, considerable gaps in research, and the need for future studies focused on certain high-risk patient populations.

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CORONARY ARTERY DISEASE (CAD) is the leading cause of death in industrialized countries. The broad clinical spectrum of CAD includes angina and myocardial infarction (MI) and is caused by atherosclerosis, a degenerative disease condition affecting the arterial vessel walls. Apart from some rare mendelian forms of CAD, most CAD is believed to have a multifactorial genetic basis involving a number of genes and environmental factors that interact to determine whether a person will develop the disease. Several types of environmental factors predicting the risk of atherosclerosis have been recognized, such as age, male sex, family history, hypertension, dyslipidemia, smoking history, and presence of diabetes mellitus. However, disentangling the genetic component of CAD remains a great challenge.

The primary area of research of genetic predispositions to CAD has focused on genes that participate in well-characterized pathophysiological pathways of the disease, such as the renin-angiotensin-aldosterone system. Several strands of evidence implicate the angiotensin I–converting enzyme (ACE) as an important modulator of atherosclerosis. Increased serum levels of ACE have been associated with CAD but, more importantly, increased ACE vascular activity has been identified in culprit lesions in acute coronary syndromes and restenotic plaques after coronary interventions. Moreover, the role of ACE inhibition in primary and secondary prevention of CAD has been well established by large clinical trials. The human ACE gene is located on chromosome 17q23. A polymorphic variant in intron 16 of this gene is characterized by an insertion (I) or a de-
letion (D) of a 287–noncoding base pair Alu repeat sequence. Early studies demonstrated a strong correlation between the D allele and levels of circulating, intracellular, and heart tissue activity of ACE.11-14 Because both alleles are considered to have codominant effects on ACE levels, individuals who are homozygous for the D allele have the highest levels of the enzyme, those homozygous for the I allele have the lowest, and heterozygous individuals have an intermediate level. Based on these effects, genotype-corrected reference values for serum ACE levels have been recently proposed, although significant overlapping between genotypes exists.15 Numerous studies have investigated the relationships between the ACE I/D gene polymorphic variant and several outcomes related to CAD, such as MI,16-20 coronary restenosis after angioplasty,21 and ischemic heart failure.22 The available evidence from the genetic association studies published to date on the association between CAD and the ACE I/D gene polymorphic variant was weak, owing to sparseness of data or disagreements among studies. Each of these studies typically involved a few cases and controls and therefore was neither adequate nor sufficiently informative to clearly demonstrate an association.23 Furthermore, the studies varied markedly by including different populations, sampling strategies, and genotyping procedures.24-26 We conducted a meta-analysis to shed some light on these contradictory results and to decrease the uncertainty of the effect size of the estimated risk.27,28 Five previously published meta-analyses on the association of ACE I/D and CAD included the relatively scarce information available at that time16-20 and failed to confirm a strong heterogeneity and the existence of potential bias.

METHODS

STUDY SELECTION

We conducted a comprehensive search of the PubMed database from its inception through February 2007. We combined search terms for ACE genotype and CAD. Search terms included ACE or angiotensin-converting enzyme; gene, polymorphism, or genetic variant; and myocardial infarction, myocardial infarct, coronary artery disease, coronary heart disease, ischemic heart disease, myocardial ischemia, angina, acute coronary syndrome, acute coronary syndromes, ACS, coronary calcification, coronary flow reserve, ischemic heart failure, heart failure, or ischemic cardiomyopathy. The retrieved studies were manually screened in their entirety to assess their appropriateness for cases and controls. When available, we recorded the frequencies of the alleles were extracted or calculated for cases and controls for the ACE I/D polymorphic variant (details available from the authors on request). The genotype distribution of cases and controls for the ACE I/D polymorphic variant (details available from the authors on request). The frequencies of the alleles were extracted or calculated for cases and controls. When available, we recorded the study population, study end point, criteria of diagnosis, demographics, matching, clinical status of controls, genotyping method, analysis for subgroups of interest (ie, individuals with diabetes mellitus, with premature disease, or at low risk and sex) (details available from the authors on request), and the genotype distribution of cases and controls for the ACE I/D polymorphic variant (details available from the authors on request). The frequencies of the alleles were extracted or calculated for cases and controls. When available, we recorded whether the genotyping in each study was blinded to clinical status. All data were extracted from published articles, and we did not contact individual authors for further information.

DATA EXTRACTION

Two investigators (E.Z. and G.K.) independently extracted data, and disagreements were resolved through consensus. The extracted data included the year of publication, ethnicity of the study population, study end point, criteria of diagnosis, demographics, matching, clinical status of controls, genotyping method, analysis for subgroups of interest (ie, individuals with diabetes mellitus, with premature disease, or at low risk and sex) (details available from the authors on request), and the genotype distribution of cases and controls for the ACE I/D polymorphic variant (details available from the authors on request). The frequencies of the alleles were extracted or calculated for cases and controls. When available, we recorded whether the genotyping in each study was blinded to clinical status. All data were extracted from published articles, and we did not contact individual authors for further information.

DATA SYNTHESIS

We performed a meta-analysis to investigate the association between ACE I/D and CAD for the allele contrast (D vs I), the recessive (DD vs ID and II), dominant (DD and ID vs II), additive (DD vs II), and codominant (II vs DD and ID) models. We calculated the overall odds ratio (OR) with the corresponding 95% confidence interval (CI) using the random effects (DerSimonian and Laird) model.31 Statistical heterogeneity across the various studies was tested with the use of the Q statistic.30,33 A P < .10 indicated a significant statistical heterogeneity across studies, allowing for the use of the random effects model.

We also performed a cumulative and recursive cumulative meta-analysis34-36 to provide a framework for updating a genetic effect from all studies and to measure how much the genetic effect changes as evidence accumulates. Thus, cumulative meta-analysis indicates the trend in estimated risk effect, and recursive cumulative meta-analysis indicates the stability in risk effect. In cumulative meta-analysis, studies were chronologically ordered by publication year, then the pooled ORs were obtained at the end of each year (ie, at each information step). In recursive cumulative meta-analysis, the relative change in pooled OR in each information step (pooled OR in the next year/ pooled OR in the current year) was calculated. A differential magnitude of effect comparing large vs small studies for the allelic contrast was verified using the Egger regression test and the Begg-Mazumdar test, based on the Kendall τ.36,37 We compared the ORs of the levels of quality characteristics (ie, consistency of levels) using the z test.

For additional analyses, the cases and controls were subgrouped on the basis of their susceptibility to MI, racial/ethnic descent, sex, age at onset (men, < 55 years; women, < 65 years),29 low risk of CAD, and the presence of diabetes mellitus (details available from the authors on request). Racial/ethnic descent was categorized into white, East Asian, black, Turkish, Latino, East Indian, and mixed population subgroups.29 However, the con-
consistency of genetic effects across these traditionally defined racial/ethnic groups does not necessarily mean that race-specific genetic effects are exactly the same.27,30

Study quality was assessed by performing subgroup or sensitivity analysis on the components of study quality that are considered important in the context of this meta-analysis.26 As quality components, the following variables could be considered: reporting of the complete genotype distribution, definition of CAD (angiographically proved CAD provides a stricter phenotype definition), age matching, genotyping procedure (genotyping with insertion-specific primers41 prevents mistyping of ID to DD and is thus considered to be more accurate compared with the originally described method by Rigat et al10), blindness of genotyping, and Hardy-Weinberg equilibrium (HWE) of the genotype distribution in the control group. Hardy-Weinberg equilibrium is a surrogate to assess study quality, and the effect of HWE is associated with problems in the design and conduct of genetic association studies.27,31 Thus, studies with controls not in HWE or studies not reporting enough information to evaluate the HWE were subjected to a sensitivity analysis.44-50 Furthermore, we performed a meta-analysis excluding all cross-sectional and cohort studies. Sources of heterogeneity were explored with the component approach by investigating the consistency between subgroups. In addition, a meta-regression procedure was adopted.27,31,60

Analyses were performed using commercially available software (StatsDirect [StatsDirect Ltd, Cheshire, England], Visual Fortran 90 [Compaq, Houston, Texas], and GLIM3.77 [Royal Statistical Society, Oxford, England]).44-50 Hardy-Weinberg equilibrium was analyzed using an exact test according to Weir.51

Table 1 presents a flowchart of retrieved studies and studies excluded, with specification of reasons. Two articles provided ethnic data on 2 separate studies each.75,150 Thus, data were obtained from 118 studies. Data were extracted by two of us (G.K. and E.Z.), and disagreements were resolved by consensus.

The studies were published from 1992 through 2007. A description of studies meeting eligibility criteria is provided in Table 1, and a list of details abstracted from the studies is available from the authors on request.

**SUMMARY STATISTICS**

The studies provided 43,733 cases with CAD and 82,606 controls free of CAD; of these, 18,139 cases had MI. In the cases and controls, the D allele was the most common. In cases and controls, the frequency of genotype ID was the highest, whereas the frequency of genotype II was the lowest. Ten studies did not provide data for all genotypes separately.* The genotype distributions are available from the authors on request.

The distribution of genotypes in the control group deviated from HWE in 15 studies,† whereas HWE deviation could not be tested for all studies.‡ Because a lack of HWE indicates possible genotyping errors and/or population stratification,44,51,166 we performed a sensitivity analysis excluding these studies. In addition, articles where HWE could not be assessed were treated as studies that deviate from HWE in the sensitivity analysis.44

**MAIN RESULTS AND SUBGROUP AND SENSITIVITY ANALYSES**

Table 2 shows the results for the association between the ACE I/D gene polymorphic variant and the risk of CAD (additional details available from the authors on request).

The main analysis for investigating the association between the D allele and the risk of CAD relative to the I allele revealed significant heterogeneity (P < .01) among the 109 studies, and the random effects pooled OR was significant (random effects OR, 1.12 [95% CI, 1.07-1.16]). The recessive and dominant models also showed significant association (random effects ORs, 1.16 [95% CI, 1.10-1.24] and 1.15 [95% CI, 1.09-1.22], respect-

*References 60, 85, 97, 107, 115, 135, 138, 147, 153, 161.
‡References 60, 85, 97, 107, 115, 135, 138, 147, 153, 161.
The additive model produced a significant association (random effects OR, 1.25 [95% CI, 1.16-1.35]) and the codominant model produced a nonsignificant association (random effects OR, 0.99 [95% CI, 0.94-1.04]) as anticipated. Thus, the ACE I/D polymorphic variant contributes to CAD susceptibility under an additive model. Exclusion of cross-sectional and population-based cohort studies did not alter the pattern of results. Studies involving only cases with MI derived the same pattern of results as that found in the main analysis in CAD.

In subgroup analysis by ethnicity (Table 3), white and Turkish subjects showed significance under an additive model with a magnitude of effects similar to that of the main analysis, whereas East Asian subjects showed significance under a recessive model for the D allele (random effects OR, 1.38 [95% CI, 1.09-1.76]). Black, Latino, and East Indian subjects showed no significant associations; however, very few studies were performed for these populations, and the results should be interpreted with caution.

In women, the ACE I/D gene polymorphic variant was not associated with susceptibility to CAD; however, in men there was a significant risk. In premature cases and in cases with low risk factors, the ACE I/D polymorphic variant was not associated with CAD, whereas in diabetic subjects there was a high risk of CAD under a recessive model (random effects OR, 1.39 [95% CI, 1.12-1.73]).

HETEROGENEITY, STUDY QUALITY, AND POTENTIAL BIAS

The cumulative meta-analysis for the allelic contrast showed a trend of association as information accumu-
In recursive cumulative meta-analysis for the allelic contrast, the relative change in the random effects ORs fluctuated around 1.00 until 1998 and then stabilized, indicating that there is sufficient evidence for investigating the association (Figure 3).

Subgroup and sensitivity analyses for the components of study quality (Figure 4) for the allelic contrast showed that exclusion of studies that violated HWE did not alter the overall results, and the same was shown for studies not reporting the complete genotype distribution. Use of an angiographically based definition of CAD and study blindness effects showed consistent results in their levels (\( P = .56 \) and \( P = .17 \), respectively); however, there was a tendency for blinded studies to produce a lower OR. Studies with age-matched controls produced nonsignificant results (random effects OR, 1.04 [95% CI, 0.97-1.12]), whereas studies with a lack of matching showed a significant association (random effects OR, 1.15 [95% CI, 1.09-1.20]) (\( P = .01 \)). Studies with insertion-specific primer genotyping produced significantly lower ORs (\( P = .01 \)) than did studies with other genotyping procedures, although both subgroup analyses derived significant associations.

The metaregression showed that the major sources of heterogeneity are racial/ethnic diversity (\( P = .01 \)) and the quality characteristics of genotyping procedure (\( P = .01 \)) and age matching (\( P = .01 \)). Use of an angiographically based definition of CAD and study blindness did not contribute significantly to overall heterogeneity (\( P = .50 \) and \( P = .41 \), respectively). Although male sex produced a significant association, the sex effect in the metaregression was not significant (\( P = .86 \)), indicating that the difference between the

### Table 2. Odds Ratios (ORs) and Heterogeneity Results for the Genetic Contrasts of ACE I/D Gene Polymorphic Variation for CAD and Subgroup Populations

<table>
<thead>
<tr>
<th>Genetic Contrast</th>
<th>Population</th>
<th>No. of Studies(^a)</th>
<th>Random Effects OR (95% CI)</th>
<th>( P ) Value(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( D vs \ I ) alleles</td>
<td>All</td>
<td>108</td>
<td>1.12 (1.07-1.16)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Case-control</td>
<td>95</td>
<td>1.12 (1.07-1.17)</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>26</td>
<td>1.07 (1.01-1.14)</td>
<td>&lt;.02</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>12</td>
<td>0.98 (0.89-1.08)</td>
<td>.26</td>
<td></td>
</tr>
<tr>
<td>Premature</td>
<td>25</td>
<td>1.06 (0.94-1.19)</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>Low risk</td>
<td>13</td>
<td>1.04 (0.89-1.21)</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>Diabetic individuals</td>
<td>8</td>
<td>1.21 (1.01-1.46)</td>
<td>.14</td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td>49</td>
<td>1.11 (1.05-1.18)</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>( DD vs \ ID ) and ( II ) genotypes</td>
<td>All</td>
<td>117</td>
<td>1.16 (1.10-1.24)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Case-control</td>
<td>101</td>
<td>1.16 (1.08-1.25)</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>30</td>
<td>1.15 (1.03-1.29)</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>15</td>
<td>0.94 (0.73-1.20)</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>Premature</td>
<td>29</td>
<td>1.08 (0.91-1.28)</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>Low risk</td>
<td>17</td>
<td>1.08 (0.95-1.37)</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>Diabetic individuals</td>
<td>10</td>
<td>1.39 (1.12-1.73)</td>
<td>.20</td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td>54</td>
<td>1.18 (1.07-1.31)</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>( DD ) and ( ID ) vs ( II ) genotypes</td>
<td>All</td>
<td>109</td>
<td>1.17 (1.09-1.25)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Case-control</td>
<td>95</td>
<td>1.17 (1.09-1.25)</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>26</td>
<td>1.09 (1.02-1.17)</td>
<td>.50</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>12</td>
<td>1.04 (0.82-1.32)</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>Premature</td>
<td>25</td>
<td>1.05 (0.91-1.21)</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>Low risk</td>
<td>13</td>
<td>1.13 (0.97-1.31)</td>
<td>.30</td>
<td></td>
</tr>
<tr>
<td>Diabetic individuals</td>
<td>8</td>
<td>1.27 (0.83-1.95)</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td>49</td>
<td>1.14 (1.05-1.24)</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>( DD ) vs ( II ) genotypes</td>
<td>All</td>
<td>108</td>
<td>1.28 (1.17-1.39)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Case-control</td>
<td>95</td>
<td>1.28 (1.17-1.39)</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>26</td>
<td>1.16 (1.04-1.28)</td>
<td>.13</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>12</td>
<td>0.99 (0.82-1.19)</td>
<td>.41</td>
<td></td>
</tr>
<tr>
<td>Premature</td>
<td>25</td>
<td>1.15 (0.95-1.41)</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>Low risk</td>
<td>13</td>
<td>1.18 (0.95-1.46)</td>
<td>.11</td>
<td></td>
</tr>
<tr>
<td>Diabetic individuals</td>
<td>8</td>
<td>1.52 (1.02-2.26)</td>
<td>.13</td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td>49</td>
<td>1.25 (1.12-1.39)</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>( ID ) vs ( DD ) and ( II ) genotypes</td>
<td>All</td>
<td>108</td>
<td>0.99 (0.94-1.04)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Case-control</td>
<td>95</td>
<td>0.99 (0.93-1.05)</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>26</td>
<td>0.98 (0.91-1.06)</td>
<td>.09</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>12</td>
<td>1.01 (0.73-1.40)</td>
<td>.01</td>
<td></td>
</tr>
<tr>
<td>Premature</td>
<td>25</td>
<td>0.98 (0.85-1.13)</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>Low risk</td>
<td>13</td>
<td>1.14 (0.91-1.43)</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>Diabetic individuals</td>
<td>8</td>
<td>0.90 (0.67-1.21)</td>
<td>.05</td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td>49</td>
<td>0.98 (0.90-1.06)</td>
<td>&lt;.01</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ACE, angiotensin-converting enzyme; CAD, coronary artery disease; CI, confidence interval; D, deletion; I, insertion; MI, myocardial infarction.
\(^a\)Includes studies not in Hardy-Weinberg equilibrium (HWE) or studies in which HWE cannot be tested.
\(^b\)Calculated by means of the \( Q \) test.
sexes can be considered marginal \( P = .98 \) and that it might be due to the limited number of studies investigating sex interaction. Other potential sources of heterogeneity could be the age at onset, differential risk exposures, and presence of diabetes mellitus.

The Egger and Begg-Mazumdar tests for the allelic contrast indicated that there is a differential magnitude of effect in large vs small studies \( P = .01 \) and \( P = .05 \), respectively.

**COMMENT**

The strength of the present analysis investigating the relationship between the ACE I/D polymorphic variant and susceptibility to CAD is based on the large amount of published data giving greater information to detect significant differences. In the present study, the consistency of genetic effects across populations from different ethnicities was investigated. The need for cumulative and recursive cumulative meta-analyses has already been highlighted.\(^{165,166}\) The stability in the relative changes in ORs indicates that there is enough evidence to draw safe conclusions about the risk effect of the ACE I/D polymorphic variant in CAD. The ACE I/D polymorphic variant is associated with an increased CAD susceptibility in certain subgroups (white, East Asian, and Turkish subjects, men, and subjects with diabetes mellitus) and the results found a modest genetic effect, with the random effects ORs ranging from 1.11 to 1.25 for the models under investigation.

Heterogeneity may result from differences in sample selection (eg, age at onset, sex, or diagnostic criteria) or in genotyping methods (2 different genotyping procedures were used), or it may be due to real differences in populations (eg, racial descent) or interactions with other unknown risk factors.\(^{167}\)

The results of the meta-analysis were affected by population origin. White and Turkish subjects showed significance under an additive model as in the main analysis, whereas East Asian subjects showed significance under a recessive model, so any conclusion should be inter-

### Table 3. Odds Ratios (ORs) and Heterogeneity Results for the Genetic Contrasts of the ACE I/D Gene Polymorphic Variant for CAD in Different Racial/Ethnic Populations

<table>
<thead>
<tr>
<th>Genetic Contrast</th>
<th>Population</th>
<th>No. of Studies</th>
<th>Random Effects OR (95% CI)</th>
<th>( P ) Value(^{a} )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D vs I alleles</strong></td>
<td>White</td>
<td>63</td>
<td>1.09 (1.04-1.14)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td></td>
<td>East Asian</td>
<td>16</td>
<td>1.14 (1.00-1.29)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>3</td>
<td>1.13 (0.92-1.39)</td>
<td>.60</td>
</tr>
<tr>
<td></td>
<td>Turkish</td>
<td>13</td>
<td>1.41 (1.21-1.64)</td>
<td>.04</td>
</tr>
<tr>
<td></td>
<td>Latino</td>
<td>3</td>
<td>1.30 (0.80-2.12)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td></td>
<td>East Indian</td>
<td>2</td>
<td>1.03 (0.79-1.35)</td>
<td>.68</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>2</td>
<td>1.01 (0.97-1.04)</td>
<td>.56</td>
</tr>
<tr>
<td><strong>DD vs ID and II genotypes</strong></td>
<td>White</td>
<td>68</td>
<td>1.11 (1.03-1.21)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td></td>
<td>East Asian</td>
<td>17</td>
<td>1.38 (1.09-1.76)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>3</td>
<td>1.32 (0.94-1.87)</td>
<td>.60</td>
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<td></td>
<td>Turkish</td>
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<td>1.47 (1.21-1.80)</td>
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<td></td>
<td>Latino</td>
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<td>1.46 (0.71-2.04)</td>
<td>.01</td>
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<tr>
<td></td>
<td>East Indian</td>
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<td>0.96 (0.66-1.39)</td>
<td>.91</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>3</td>
<td>1.01 (0.96-1.06)</td>
<td>.99</td>
</tr>
<tr>
<td><strong>DD and ID vs II genotypes</strong></td>
<td>White</td>
<td>64</td>
<td>1.15 (1.07-1.24)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td></td>
<td>East Asian</td>
<td>16</td>
<td>1.10 (0.95-1.28)</td>
<td>.02</td>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>3</td>
<td>1.07 (0.77-1.49)</td>
<td>.86</td>
</tr>
<tr>
<td></td>
<td>Turkish</td>
<td>13</td>
<td>1.70 (1.29-2.24)</td>
<td>.10</td>
</tr>
<tr>
<td></td>
<td>Latino</td>
<td>3</td>
<td>1.40 (0.69-2.84)</td>
<td>.04</td>
</tr>
<tr>
<td></td>
<td>East Indian</td>
<td>2</td>
<td>1.07 (0.72-1.58)</td>
<td>.75</td>
</tr>
<tr>
<td></td>
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<td>2</td>
<td>1.01 (0.90-1.12)</td>
<td>.31</td>
</tr>
<tr>
<td><strong>DD vs II genotypes</strong></td>
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<td>63</td>
<td>1.21 (1.11-1.33)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td></td>
<td>East Asian</td>
<td>16</td>
<td>1.32 (1.03-1.69)</td>
<td>&lt;.01</td>
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<tr>
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<td>Black</td>
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<td>1.26 (0.82-1.96)</td>
<td>.72</td>
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<td>1.97 (1.47-2.63)</td>
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<tr>
<td></td>
<td>Latino</td>
<td>3</td>
<td>1.70 (0.60-4.78)</td>
<td>.01</td>
</tr>
<tr>
<td></td>
<td>East Indian</td>
<td>2</td>
<td>1.04 (0.63-1.72)</td>
<td>.70</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>2</td>
<td>1.01 (0.94-1.08)</td>
<td>.42</td>
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<tr>
<td><strong>ID vs DD and II genotypes</strong></td>
<td>White</td>
<td>63</td>
<td>1.01 (0.94-1.08)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td></td>
<td>East Asian</td>
<td>16</td>
<td>0.94 (0.85-1.03)</td>
<td>.51</td>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>3</td>
<td>0.87 (0.66-1.16)</td>
<td>.69</td>
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<tr>
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<td>0.91 (0.74-1.13)</td>
<td>.05</td>
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<td></td>
<td>Latino</td>
<td>3</td>
<td>0.99 (0.63-1.55)</td>
<td>.08</td>
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<tr>
<td></td>
<td>East Indian</td>
<td>2</td>
<td>1.07 (0.73-1.56)</td>
<td>.98</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>2</td>
<td>0.99 (0.95-1.04)</td>
<td>.50</td>
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</table>

**Abbreviations:** ACE, angiotensin-converting enzyme; CAD, coronary artery disease; CI, confidence interval; D, deletion; I, insertion.

\(^{a}\)Calculated by means of the \( Q \) test.
interpreted with caution. Nevertheless, in East Asian subjects, the frequency of the DD genotype is lower than that in white subjects.\textsuperscript{169} This lower frequency of the DD genotype, with the small numbers of subjects enrolled in most studies in East Asian subjects, implies that any negative conclusion could be due to a low statistical power. The rest of the examined racial/ethnic groups showed no significant effect. Only 3 studies were conducted in black subjects, although this population is disproportionately affected by heart disease. The differences in effects of the ACE I/D polymorphic variant in the examined racial/ethnic groups could reflect true race-specific genetic effects, because functional analyses of variation in the ACE gene have indicated different quantitative trait loci in particular racial/ethnic groups.\textsuperscript{170-172}

The differential association in men and women provides an intriguing finding of sex-specific effects for the ACE I/D polymorphic variant that have not been reported in previous meta-analyses.\textsuperscript{16-20} Overall, 11 studies have investigated a potential sex interaction for the ACE I/D polymorphic variant in CAD risk.\textsuperscript{§} Six studies report a positive association restricted only to men,\textsuperscript{52,58,64,97,147} 4 studies did not find any association for both sexes,\textsuperscript{80,82,104,113}, and 1 study identified a positive association for men and women.\textsuperscript{135} Although not consistent, such a sex-specific influence could result from the effect of corticosteroid hormones, which affect the activity of the renin-angiotensin-aldosterone system in a variety of tissues.\textsuperscript{173} The incorporation of sex-dependent models in future studies is awaited to provide a more powerful analytical framework.\textsuperscript{174}

We performed subgroup analyses for premature cases and low-risk individuals. These subgroups are of specific interest, because genetic factors may have greater contribution in those in whom CAD develops at a younger age and in the absence of strong environmental risk factors.\textsuperscript{175,176} Although the definition of low-risk subgroups was not similar in the analyzed studies (details available from the authors on request), these subgroups consisted mostly of individuals with a low body mass index and a nondyslipidemic profile. Contrary to what was anticipated, the subgroup analyses produced no significant results, making the robustness of the main analysis debatable. The positive association of the ACE I/D polymorphic variant found in subgroup analysis for diabetic subjects concurred with that of previous reports.\textsuperscript{177}

The subgroup analysis involving only cases with MI had results similar to those of the main pooled analysis. By definition, CAD represents a broad clinical entity and

\textsuperscript{§}References 52, 58, 64, 80, 82, 96, 97, 104, 113, 133, 147.
is probably a suboptimal end point for genetic association studies, where the definition of phenotypes is of crucial importance. Consequently, more distinct and precise phenotypes such as MI (under the World Health Organization criteria) or a more standardized definition of CAD (under strict angiographic or intravascular ultrasonographic criteria) should be used in future studies.

Our main analysis was based on unadjusted estimates. However, a more precise analysis could be performed if adjusted estimates were available in all studies. Another potentially important limitation of our analysis is that our results were not adjusted for the use of ACE inhibitors by the cases and/or the controls because such information was not universally provided by the studies. Because recent data indicate that the response to ACE inhibitors could depend on the ACE genotype, this lack of correction might have influenced our results.

Most of the analyzed studies were of a case-control design and had a retrospective character. This means that a significant proportion of cases were recruited some time (and at varying times) after an incident MI. About half of the deaths due to an acute MI occur in the first few hours, typically before admission to a hospital. If the risk genotype is associated with a poor prognosis immediately after MI, then this genotype will be underrepresented in cases at the time of enrollment. Prospective studies could overcome such a confounding factor. However, such a claim is controversial, in consideration of the evidence linking the D allele to human longevity and sudden cardiac death.

The relevant methodological aspects of the studies and the influence of quality were assessed individually on the basis of a subgroup or a sensitivity analysis. Although composite quality scales may provide an overall assessment when comparing studies, the use of such scales can be problematic, and the interpretation of results is difficult. However, there is some controversy in the literature as to whether variations in study quality constitute an important source of heterogeneity. The only predominant measures of quality affecting heterogeneity could be the method of genotyping (the use of insertion-specific primers is considered to be a more accurate method) and the age matching.

Prevalence of CAD increases gradually with age, and CAD is very common in the aging population. Future studies should select cases with very-early-onset disease and a strong family history or a strong genetic etiology, and this approach could enhance the statistical power to detect a genetic effect. However, in studies investigating premature CAD, the controls had a relatively younger mean age. The absence of CAD in young patients does not exclude the possibility of CAD developing in the future. Therefore, a control group may include subjects who are still at risk of CAD.

For most meta-analysis applications in genetics and genomics, the sample sizes of individual studies tend to be small. The power of single studies is usually very low. A combination of low power and high biological multiplicity results is expected to result in a very high rate of false discovery. Synthesis of data from many studies is expected to improve power and reduce the rate of false discovery in all circumstances, and the gain could be considerable unless there is very large genuine between-study heterogeneity. However, power calculations are usually considered inappropriate in meta-analysis, because those data are already assembled. Although this meta-analysis was based on a large number of subjects, the investigation of genetic associations should be based on large population studies with similar study designs and standardized case and control definitions. Winkelmann et al suggested the creation of large databases, which will facilitate the sharing of resources among investigators. This will result in the generation of fewer but more reliable studies, helping to mitigate the problem of the publication of multiple investigations involving small numbers of patients, which often produce confounding results. Individual researchers should also publish or make easily available sufficient information to facilitate future meta-analysis, including relevant genotype, phenotype (such as MI cases or angiographic data), and subgroup data (eg, sex, risk factors, and age at onset). Future studies investigating the role of the ACE I/D polymorphic variant in CAD should be planned with the idea of being incorporated with other similar studies in an update meta-analysis.

Because the ACE I/D polymorphic variant is intronic, it is unlikely to be functional. Despite considerable effort, the precise location of the functional polymorphic variant or variants is still unknown. In white subjects, there are 3 major haplotypes covering 90% of the gene variation that exhibit strong linkage disequilibrium with the I/D polymorphic variant. Within populations of African origin, a great variety of polymorphic variants is found, and serum ACE levels are not linked to the I/D variant. A cohort study in Africans demonstrated that a single nucleotide polymorphism in exon 17 and an additional one in the upstream untranslated region are responsible for the variation in ACE serum activity. Because a functional variation in the ACE gene has yet to be completely characterized, future studies using the HapMap tagging single nucleotide polymorphism data could provide useful insights regarding the disease-associated gene haplotypes.

In addition, other probable genetic risk factors interacting with this polymorphic variant should be investigated. The presence of epistatic loci (ie, the effect of one locus is altered or masked by effects at another locus) has been investigated by few studies, to date. Elucidating the genetic contribution of the renin-angiotensin-aldosterone system to CAD would demand investigation of association for many variants of genes that constitute this pathophysiological pathway. Moreover, many environmental factors have been associated with increased risk of CAD. Despite difficulties in study design and in the assessment of environmental exposures, such factors should be incorporated in future studies to precisely define the role of specific genetic variants and the relative impact of genes and environment on the distribution of the phenotype. Therefore, this meta-analysis could be a guide for future case-control studies investigating the genetic basis of CAD.

In conclusion, the present meta-analysis supported an association between the ACE I/D polymorphic variant and...
CAD. In the main analysis, the ACE I/D polymorphic variant was found to contribute to CAD susceptibility under an additive model, whereas, in subgroup analyses, the pattern of results was heterogeneous. However, 15 years after the first publication and despite the undertaking of more than 100 genetic association studies designed to test this hypothesis, the exact role of the variation in the human genome most studied to date remains an unresolved issue.

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Author Contributions: Study concept and design: Zintzaras, Raman, Kitsios, and Lau. Acquisition of data: Zintzaras and Kitsios. Analysis and interpretation of data: Zintzaras and Kitsios. Drafting of the manuscript: Zintzaras and Kitsios. Critical revision of the manuscript for important intellectual content: Zintzaras, Raman, Kitsios, and Lau. Statistical analysis: Zintzaras, Kitsios, and Lau. Administrative, technical, and material support: Zintzaras, Raman, and Kitsios. Study supervision: Zintzaras and Lau.

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Nissen SE. Application of intravascular ultrasound to characterize coronary artery disease and assess the progression or regression of atherosclerosis. Am J Cardiol. 2002;89(4A):24B-31B.


Errors in Byline and Author Affiliations: In the Original Investigation titled “Lipoprotein(a) Levels and Risk of Future Coronary Heart Disease: Large-Scale Prospective Data” by Bennet et al, published in the March 24 issue of the Archives (2008;168[6]:598-608), errors occurred in the byline and Author Affiliations on page 598. In the byline, the fourth author’s name should have appeared as “Gudny Eiriksdottir, MSc.” In the Author Affiliations on the same page, the second affiliation should have appeared as “Icelandic Heart Association, Kopavogur, Iceland (Ms Eiriksdottir and Drs Sigurdsson and Gudnason).” This article was corrected online for typographical errors on March 24, 2008.