Angiotensin I-Converting Enzyme Insertion/Deletion Polymorphism and Cardiac Mortality and Morbidity After Coronary Artery Bypass Graft Surgery*

Henry Völzke, MD; Julia Engel, MD; Volker Kleine, MD; Christian Schwahn, PhD; Johannes B. Dahm, MD; Lothar Eckel, MD; and Rainer Rettig, MD

Study objectives: This study was designed to evaluate whether the insertion (I)/deletion (D) polymorphism of the angiotensin I-converting enzyme (ACE) gene is associated with mortality and cardiac morbidity after coronary artery bypass graft surgery (CABG).

Methods and results: The ACE I/D genotype was determined in 249 consecutive patients who underwent CABG. Follow-up information (after 2 years) was obtained in 247 patients (99.2%). The primary end point was total mortality; the secondary end point was mortality from cardiac reasons, or the need for myocardial revascularization (coronary angioplasty or recurrent CABG) during follow-up. At follow-up, total mortality was 9.7% (all patients). None of the 51 patients with the ACE II genotype, 14 of 125 patients with the ACE ID genotype (11.2%), and 10 of 71 with the ACE DD genotype (14.1%) died during follow-up (p < 0.05). The ACE DD genotype, older age, diabetes mellitus, decreased left ventricular ejection fraction, and lack of internal mammary artery graft were independently related to an increased mortality after CABG. The incidence of the secondary end point was 14.5% (all patients): ACE II, 5.8%; ACE ID, 9.4%; ACE DD, 30.3% (p < 0.05). The ACE DD genotype and the presence of a left main coronary artery stenosis ≥ 50% were independent predictors for the secondary end point.

Conclusion: The ACE DD genotype is associated with increased midterm mortality and cardiac morbidity after CABG. (CHEST 2002; 122:31–36)

Key words: angiotensin-converting enzyme insertion/deletion genotype; cardiac morbidity; coronary artery bypass graft surgery; mortality

Abbreviations: ACE = angiotensin I-converting enzyme; CABG = coronary artery bypass graft surgery; CAD = coronary artery disease; CI = confidence interval; D = deletion; exp[β] = β exponent; I = insertion; PCR = polymerase chain reaction

The haptoglobin 2–2 genotype has been shown to be associated with a shortened graft survival time in patients who underwent recurrent coronary artery bypass graft surgery (CABG).1 This finding points toward a role of genetic factors as determinants of coronary artery bypass graft survival. Until now, the relationships among genetic factors, mortality, and cardiac morbidity after CABG have not been addressed in the literature.

Rigat et al2 first described an insertion (I)/deletion (D) polymorphism in intron 16 of the angiotensin I-converting enzyme (ACE) gene. Carriers of the DD genotype show higher plasma ACE levels compared to individuals with the II or ID genotypes.2 The I/D polymorphism accounts for approximately 50% of the variance of the plasma ACE levels.2 Moreover, the DD genotype is associated with elevated ACE activities in plasma3 and cardiac tissue.4 Relationships among the ACE DD genotype and an increased risk for coronary artery disease (CAD),3,5 acute myocardial infarction,6,7 and left ventricular hypertrophy,8,9 have been described. The association between the ACE DD genotype and an increased risk for cardiovascular disorders appears to be particularly prominent in patients who are at a low cardiovascular risk, as judged by the presence or absence of conventional risk factors,3,6 although these findings have not always been confirmed.10,11

*From the Medical Department B (Drs. Völzke, Engel, Kleine, and Dahm), Department of Biostatistics (Dr. Schwahn), and Department of Physiology (Dr. Rettig), Ernst Moritz Arndt University Greifswald, Greifswald; and Center of Cardiology (Dr. Eckel), Karlsburg, Germany.

Manuscript received March 20, 2001; revision accepted February 12, 2002.

Correspondence to: Henry Völzke, MD, Clinic of Internal Medicine B, Ernst Moritz Arndt University, Friedrich-Loeffler-Strasse 23a, D-17487 Greifswald, Germany; e-mail: voelzke@mail.uni-greifswald.de
The aim of this study was to prospectively investigate the relationships among the ACE I/D genotype, mortality, and cardiac morbidity in patients undergoing CABG.

**Materials and Methods**

**Study Population**

The study population comprised 249 consecutive patients with single or multivessel CAD who were recruited on the day before they underwent CABG, between May 1996 and August 1997. None of the patients had undergone cardiac surgery before. The study was approved by the institutional review committee of the University of Greifswald. Prior to inclusion in the study, all subjects gave informed consent. All patients were residents of Western Pomerania, a rural area located in northeast Germany. Clinical, laboratory, and angiographic data were obtained from medical records.

**CABG Procedure**

After induction of anesthesia, surgery was performed in cardioplegia, moderate hypothermia, and hemodilution. Heparin was administered (2 to 3 mg/kg of body weight) before the internal mammary artery or the saphenous veins were harvested. Patients were operated on through a midline sternotomy. All patients underwent aortic cross-clamping. The number of grafts that were necessary to achieve complete revascularization in each individual patient was determined by the surgeon at the time of operation. The median number of grafts per patient was three (range, one to five grafts). Two hundred thirty-seven internal mammary artery grafts were used in 221 of the 249 patients.

**Follow-up**

Information on survival status, date of death, recurrent percutaneous transluminal coronary angioplasties, or recurrent CABG procedures was obtained from phone calls with family doctors. Whenever necessary, the patient, his or her relatives, and local hospitals were contacted for further information. The current vital status remained unknown in two patients (0.8%). The hospitals were contacted for further information. The current vital status remained unknown in two patients (0.8%). The cumulative follow-up time was 464.7 patient-years (median follow-up period, 706 days; range, 2 to 987 days).

**Definitions of End Points**

The primary end point was total mortality; data for all patients with a complete follow-up (n = 247) were analyzed. The secondary end point was mortality from cardiac reasons (myocardial infarction, heart failure, and sudden death) or need for recurrent myocardial revascularization (percutaneous transluminal coronary angioplasty or recurrent CABG) during follow-up.

**Genetic Analysis**

Genetic analysis was performed by laboratory personnel who were blinded for the clinical data. Genomic DNA was extracted from peripheral blood leukocytes using a commercially available kit (High Pure PCR Template Preparation Kit; Boehringer Mannheim; Mannheim, Germany). The ACE I/D genotype was determined by polymerase chain reaction (PCR) as described by Rigat et al, with the modification that dimethylsulphoxide was added to the PCR mixture at a final concentration of 5% (volume/volume). The inclusion of 5% dimethylsulphoxide in the reaction mixture has been shown to improve the amplification of the I allele in I/D heterozygotes. To further safeguard against ID vs DD mistyping, the DNA from all patients initially typed DD was subjected to a second PCR analysis with a sense primer from the 5' end of the insertion sequence, along with the standard antisense primer. PCR products were subjected to electrophoresis in 2% agarose gels and visualized by ethidium bromide staining.

**Statistics**

Data on quantitative characteristics are expressed as median and range. Data on qualitative characteristics are expressed as percentage values or absolute numbers as indicated. Patients were classified into three groups according to their ACE I/D genotypes. Biometric analyses for the secondary end point revealed a necessary sample size of 89 patients to detect a 15% difference (group 1 proportion \( \pi_1 = 0.95 \); group 2 proportion \( \pi_2 = 0.80 \)) on a one-sided test significance level of \( \alpha = 0.05 \), with a statistical power of 80% for equal groups. Since we expected unequal group sizes (the D/D genotype is less frequent than the I/I and I/D genotypes combined), we introduced a correction factor \( f \) of 1.33, calculated according to the following formula:

\[
f = \frac{(1 + r)^2}{4 \times r}
\]

where \( r = n_1/n_2 \) and \( n_1 \) and \( n_2 \) are the percentages of patients expected to fall in each group. Expecting that approximately 75% of our patients would have the I/I or D/D genotype \( (n_1 = 75\%) \) and 25% would have the D/D genotype \( (n_2 = 25\%) \), it follows that \( r = 3 \) and:

\[
f = \frac{(1 + 3)^2}{4 \times 3} = 1.33
\]

With this correction factor, the calculated total study population for the two groups amounts to 89 \( \times 1.33 \times 2 = 237 \) patients. We used one-sided testing because the D/D genotype was reported to be associated with a higher cardiovascular risk. A lower cardiovascular risk in patients with the D/D genotype would not be biologically plausible.

Comparisons between groups were made using \( \chi^2 \) test (nominal data) or Kruskal-Wallis H test (interval data). The cumulative survival and the cumulative event-free survival were calculated by Kaplan-Meier analysis. The comparison of ACE I/D genotype-specific survival curves were done by log-rank test. The analysis of independent predictors for the primary and secondary end points were performed with Cox regression analysis with estimation of significances by Wald statistics. In addition, the \( \beta \) exponent \( (\exp(\beta)) \) was calculated, and values are given with the lower and upper 95% confidence interval (CI). Partial correlation was used to evaluate the contribution of individual characteristics on the variability of end points. A value of \( p < 0.05 \) was considered statistically significant. Cox regression analyses were performed using software (SPSS COXREG; SPSS GmbH Software; Munich, Germany) with the Collinearity Diagnostics option and assistance from SPSS EXPLORE (SPSS GmbH Software) for evaluation of regression assumptions. All statistical assumptions were met, and no multicollinearity problems were found in our analysis. Biometric analyses were performed with study planning software (nQuery; Statistical Solutions; Cork, Ireland).
RESULTS

Baseline Patient Characteristics

The three ACE I/D genotype groups did not differ with respect to baseline clinical characteristics such as age, sex, or body mass index. However, the ACE I/D polymorphism was associated with systolic and diastolic BP (Table 1). Data on angiographic characteristics and operation details (Table 2) were similar in the three genotype groups. The length of hospital stay also did not differ among the three genotype groups: II genotype, 10 days (range, 8 to 19 days); ID genotype, 11 days (range, 8 to 29 days); and DD genotype, 10 days (range, 7 to 33 days) [p = 0.52]. The distribution of the ACE I/D genotypes was compatible with the Hardy-Weinberg disequilibrium.

Primary End Point

The 2-year total mortality rate after CABG was 9.7% (Fig 1). Eleven patients died from cardiac reasons, and 13 patients died from noncardiac reasons (Table 3). Cox regression analysis revealed the reasons, and 13 patients died from noncardiac rea-

9.7% (Fig 1). Eleven patients died from cardiac

Table 1—Clinical Characteristics of Patients With Different ACE Genotypes at Baseline*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>ACE I/I Genotype (n = 51)</th>
<th>ACE I/D Genotype (n = 125)</th>
<th>ACE D/D Genotype (n = 71)</th>
<th>p Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>65.7 (40.9–81.9)</td>
<td>63.8 (42.6–86.2)</td>
<td>65.1 (37.6–77.2)</td>
<td>0.48</td>
</tr>
<tr>
<td>Male sex</td>
<td>41 (80.4)</td>
<td>101 (80.8)</td>
<td>60 (84.5)</td>
<td>0.78</td>
</tr>
<tr>
<td>Body mass index</td>
<td>27.9 (20.7–39.7)</td>
<td>27.6 (19.1–39.9)</td>
<td>28.1 (20.3–40.8)</td>
<td>0.77</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.9 (0.3–8.0)</td>
<td>2.2 (0.7–7.6)</td>
<td>2.0 (0.9–9.9)</td>
<td>0.07</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.5 (3.2–9.0)</td>
<td>6.1 (3.0–12.6)</td>
<td>6.2 (4.2–15.2)</td>
<td>0.41</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.5 (1.5–6.9)</td>
<td>3.8 (1.4–7.0)</td>
<td>3.8 (1.9–12.5)</td>
<td>0.73</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.0 (0.4–3.8)</td>
<td>1.0 (0.5–2.1)</td>
<td>1.0 (0.4–1.8)</td>
<td>0.88</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>130 (90–190)</td>
<td>120 (90–150)</td>
<td>130 (100–160)</td>
<td>&lt; 0.01†</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>80 (60–100)</td>
<td>80 (50–110)</td>
<td>80 (60–100)</td>
<td>0.01†</td>
</tr>
<tr>
<td>Hypertension</td>
<td>34 (66.7)</td>
<td>69 (55.2)</td>
<td>46 (64.8)</td>
<td>0.24</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>16 (31.4)</td>
<td>43 (34.4)</td>
<td>17 (23.9)</td>
<td>0.31</td>
</tr>
<tr>
<td>Smoking (current)</td>
<td>9 (18.0)</td>
<td>29 (23.6)</td>
<td>18 (25.7)</td>
<td>0.60</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>19 (37.3)</td>
<td>42 (33.6)</td>
<td>18 (36.6)</td>
<td>0.60</td>
</tr>
<tr>
<td>HMG CoA reductase inhibitors</td>
<td>48 (94.1)</td>
<td>117 (93.6)</td>
<td>68 (93.8)</td>
<td>0.71</td>
</tr>
<tr>
<td>Aspirin</td>
<td>47 (92.2)</td>
<td>120 (96.0)</td>
<td>62 (97.3)</td>
<td>0.23</td>
</tr>
</tbody>
</table>

*Data are presented as mean (range) or No. (%). LDL = low-density lipoprotein; HDL = high-density lipoprotein; HMG CoA = 3-hydroxy-3-

methylglutaryl coenzyme A.

†χ² test (nominal data) or Kruskal-Wallis H test (interval data).

†Indicates significance.

www.chestjournal.org
cardiac mortality and morbidity after CABG. Although 54% of all deceased patients died from noncardiac reasons (Table 3), the ACE I/D genotype explained 10.7% of the total mortality. To further analyze the association between this genetic factor and the risk for cardiac events we defined the secondary end point as a combination of cardiac death and coronary reintervention. The ACE I/D genotype was the main risk factor for the secondary end point, explaining 17.7% of cardiac events within the 2-year follow-up period after CABG.

As illustrated in Figure 1, much of the total mortality occurred during the early postoperative period. We therefore reanalyzed our data deleting those patients who died during the first 30 days after CABG. Under these circumstances, the ACE I/D genotype was no longer statistically associated with total mortality. However, the ACE I/D genotype remained the only independent predictor of cardiac mortality and morbidity. These data indicate that the ACE I/D genotype is associated with the midterm cardiac but not the noncardiac risk after CABG.

Table 2—Angiographic Characteristics and Operation Details of Patients With Different ACE Genotypes at Baseline

<table>
<thead>
<tr>
<th>Variables</th>
<th>ACE I/I Genotype</th>
<th>ACE I/D Genotype</th>
<th>ACE D/D Genotype</th>
<th>p Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 51)</td>
<td>(n = 125)</td>
<td>(n = 71)</td>
<td></td>
</tr>
<tr>
<td>Left ventricular wall motion disturbance</td>
<td>37 (72.5)</td>
<td>98 (78.4)</td>
<td>51 (71.8)</td>
<td>0.52</td>
</tr>
<tr>
<td>Left ventricular ejection fraction, %</td>
<td>50 (97.3)</td>
<td>50 (20-77)</td>
<td>55 (20-81)</td>
<td>0.37</td>
</tr>
<tr>
<td>Left main coronary artery stenosis ≥ 50%</td>
<td>10 (19.6)</td>
<td>13 (19.4)</td>
<td>5 (7.0)</td>
<td>0.09</td>
</tr>
<tr>
<td>Left anterior descending artery stenosis ≥ 50%</td>
<td>49 (96.1)</td>
<td>115 (92.0)</td>
<td>63 (88.7)</td>
<td>0.34</td>
</tr>
<tr>
<td>Circumflex artery stenosis ≥ 50%</td>
<td>42 (82.4)</td>
<td>104 (83.2)</td>
<td>59 (83.1)</td>
<td>0.99</td>
</tr>
<tr>
<td>Right coronary artery stenosis ≥ 50%</td>
<td>46 (90.2)</td>
<td>107 (85.6)</td>
<td>71 (80.3)</td>
<td>0.31</td>
</tr>
<tr>
<td>Urgent procedure status</td>
<td>2 (3.9)</td>
<td>10 (8.0)</td>
<td>5 (7.0)</td>
<td>0.62</td>
</tr>
<tr>
<td>No. of grafts</td>
<td>3 (1–5)</td>
<td>3 (1–5)</td>
<td>3 (1–5)</td>
<td>0.64</td>
</tr>
<tr>
<td>Use of internal mammary graft</td>
<td>46 (90.2)</td>
<td>111 (88.8)</td>
<td>64 (90.1)</td>
<td>0.94</td>
</tr>
</tbody>
</table>

*Data are presented as No. (%) or mean (range).
†χ² test (nominal data) or Kruskal-Wallis H test (interval data).

The analysis of baseline characteristics revealed an association between the ACE I/D polymorphism and systolic and diastolic BPs, respectively. The possible association between the ACE I/D genotype and BP or hypertension is still under debate. Some groups reported a relationship, whereas other groups failed to find such an association, and yet other groups reported a relationship, whereas other groups failed to find such an association, and yet other groups reported a relationship.

Table 3—Causes of Death After CABG Procedure

<table>
<thead>
<tr>
<th>Causes of Death</th>
<th>Total</th>
<th>ACE I/H Genotype (n = 51)</th>
<th>ACE I/D Genotype (n = 125)</th>
<th>ACE D/D Genotype (n = 71)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatal myocardial infarction</td>
<td>8 (3.2)</td>
<td>0</td>
<td>5 (6.2)</td>
<td>3 (2.1)</td>
</tr>
<tr>
<td>Heart failure</td>
<td>2 (0.8)</td>
<td>0</td>
<td>2 (2.5)</td>
<td>0</td>
</tr>
<tr>
<td>Sudden death</td>
<td>1 (0.4)</td>
<td>0</td>
<td>0</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>Postoperative sepsis</td>
<td>4 (1.6)</td>
<td>0</td>
<td>1 (1.2)</td>
<td>3 (2.1)</td>
</tr>
<tr>
<td>Malignant disease</td>
<td>3 (1.2)</td>
<td>2 (2.5)</td>
<td>1 (0.7)</td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>2 (0.8)</td>
<td>0</td>
<td>2 (2.5)</td>
<td>0</td>
</tr>
<tr>
<td>Suicide</td>
<td>2 (0.8)</td>
<td>0</td>
<td>1 (1.2)</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>Ischemic cerebral infarction</td>
<td>1 (0.4)</td>
<td>0</td>
<td>0</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>Intracerebral bleeding</td>
<td>1 (0.4)</td>
<td>0</td>
<td>1 (1.2)</td>
<td>0</td>
</tr>
</tbody>
</table>

*Data are presented as No. (%).
groups reported a context-dependent association. The cohort investigated in the present study was recruited from consecutive patients with severe CAD. BP in this cohort may be influenced by several disease- and treatment-related factors. Thus, our study does not allow us to draw reliable conclusions with respect to the relationship between the ACE I/D genotype and BP.

The mechanism by which the ACE I/D polymorphism may affect mortality and morbidity after CABG is unclear. There is evidence that the renin-angiotensin system is involved in the pathogenesis of atherosclerosis and in the remodeling of venous bypass grafts. Thus, angiotensin II causes vasoconstriction, activates matrix synthesis, and causes an enhanced expression of proto-oncogen messenger RNA. Angiotensin II also stimulates the proliferation of cultured vascular smooth-muscle cells, accelerates the synthesis of platelet-derived growth factor in vascular smooth-muscle cells, and causes hypertrophic changes of the vessel wall. Tissue concentrations of ACE are elevated in the endothelial layer and in the intimal hyperplasia of experimental vein grafts. We conclude that the ACE I/D genotype is associated with cardiac mortality and morbidity after CABG.

REFERENCES


Figure 2. Cumulative cardiac event-free survival after CABG in all patients and with respect to ACE I/D genotypes.

- ACE-II
- ACE-ID
- ACE-DD
29 Turner ST, Boerwinkle E, Sing CF. Context-dependent associations of the ACE I/D polymorphism with blood pressure. Hypertension 1999; 34:773–778