Angiotensin I–Converting Enzyme and Plasminogen Activator Inhibitor-1 Gene Variants: Risk of Mortality and Fatal Cardiovascular Disease in an Elderly Population-Based Cohort

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OBJECTIVES
We studied the contribution of putative risk genotypes at the angiotensin I–converting enzyme inhibitor (ACE D/D) and plasminogen activator inhibitor-1 (PAI-1 4G/4G) loci to all-cause and cardiovascular mortality in a population-based cohort.

BACKGROUND
The ACE D/D and PAI-1 4G/4G genotypes have been consistently associated with elevated plasma activities of the gene products. Their role in cardiovascular disease, although explored intensively, is still equivocal.

METHODS
The ACE and PAI-1 genotypes were determined in 648 subjects ≥85 years old. In a cross-sectional analysis, the genotype distributions in a subset of 356 elderly subjects who were born in Leiden, The Netherlands, were compared with those in 250 young subjects whose families originated from the same geographic region. In addition, the complete cohort of elderly subjects was followed over 10 years for all-cause and cardiovascular mortality and was stratified according to genotype.

RESULTS
In the cross-sectional analysis, the ACE and PAI-1 genotype distributions were similar in elderly and young subjects. In the prospective follow-up study, however, the age-adjusted risk of fatal ischemic heart disease was increased threefold (95% confidence interval [CI] 1.2 to 7.6) in elderly men carrying the PAI-1 4G/4G genotype. The risk of all-cause mortality was not increased among elderly subjects carrying the PAI-1 4G/4G (relative risk [RR] 0.9, 95% CI 0.7 to 1.1) or the ACE D/D genotype (RR 0.9, 95% CI 0.7 to 1.1), nor did we observe elevated risks of death from all cardiovascular diseases combined. There was no interaction between the genotypes.

CONCLUSIONS
The PAI 4G/4G genotype may be a risk factor for fatal ischemic heart disease in elderly men. The impact of moderately increased ACE and PAI-1 activities associated with the ACE D/D and PAI-1 4G/4G genotypes is too small to affect mortality in the general population. (J Am Coll Cardiol 1999;34:1176–83) © 1999 by the American College of Cardiology

Over the last years a number of common gene variants have been identified that are associated with plasma levels of the gene products and the risk of cardiovascular disease. Most studies performed thus far have not addressed the association of these variants with fatal cardiovascular disease or have only included a small number of fatal cases. Also, very few studies have yet explored how combinations of possible risk genotypes affect disease risk. Establishing such genetic risk profiles is only feasible when relatively large populations are available and the putative risk alleles have a high frequency in the general population. Two highly frequent genetic variants that may be linked to cardiovascular disease risk have been identified in the genes encoding angiotensin I–converting enzyme (ACE) inhibitor and plasminogen activator inhibitor-1 (PAI-1).

ACE catalyzes the conversion of angiotensin I to angiotensin II, which is a potent vasoconstrictor and promotes vascular smooth muscle cell growth and degrades bradykinin, which is a vasodilator. The ACE insertion/deletion (I/D) polymorphism arises from the presence or absence of
an alu repeat located in intron 16 of the ACE gene (frequency of ACE D-allele in whites ≈0.54) (1). The ACE D-allele is associated with increased ACE activity in plasma (1,2) and tissue (3). Several studies have reported an increased frequency of the ACE D/D genotype in survivors of myocardial infarction as compared with healthy subjects (4–7). However, these results have been challenged by the negative findings from other studies (8–15). In addition to the ongoing debate on the deleteriousness of the ACE D/D genotype, a beneficial influence on survival in very old age was suggested by the increased frequency of the ACE D/D genotype in French centenarians (16).

The primary inhibitor of fibrinolysis is PAI-1. A 4/5-guanine-tract (4G/5G) polymorphism was identified in the promoter of the PAI-1 gene 675 base pairs upstream from the start of transcription (17). The PAI-1 4G allele is associated with elevated PAI-1 levels in plasma (frequency of PAI-1 4G-allele in whites ≈0.52) (17–19). This association is especially strong among subjects with relatively high plasma levels of triglycerides, insulin or glucose (20,21).

Studies in vitro indicate that the 4G-allele is unable to bind a repressor and is associated with increased transcription of the PAI-1 gene (17,18). In addition, a very low density lipoprotein responsive element was found to partly overlap the guanine tract, which may provide a molecular explanation for the modification effect by triglycerides (22). The elevated plasma PAI-1 level in 4G/4G homozygotes may result in an increased risk of coronary heart disease as a consequence of a diminished fibrinolytic capacity. Studies investigating this hypothesis, however, have produced conflicting results (18,19,23–26).

If a deleterious effect of the ACE D-allele and the PAI-1 4G-allele is present, carriers of both risk alleles may be especially susceptible to disease owing to the link that exists between the renin–angiotensin system and fibrinolytic function. Infusion of angiotensin II increases plasma PAI-1 activity in humans (27), and recently, two studies showed that the ACE D/D genotype was associated with elevated PAI-1 levels in plasma (28,29). Hence, the ACE and PAI-1 polymorphisms may be involved in cardiovascular disease in part by the same etiologic pathway.

The aim of the present study was to assess whether the ACE and PAI-1 polymorphisms, either separately or in combination, are associated with mortality in the general population. This was done using two designs within a population-based study among subjects ≥85 years old (Leiden 85-plus study [30]). The influence of the gene variants on mortality before the age of 85 years was studied in a cross-sectional design by comparing the genotype distributions in Leiden-born subjects ≥85 years old with those in young subjects whose families originated from the same geographic region (31). The relation of the gene variants to all-cause and cardiovascular mortality above the age of 85 years was investigated in a prospective study with a 10-year follow-up period using the complete elderly cohort. During follow up, the all-cause mortality rate was 89% and the cardiovascular mortality rate was 38%.

METHODS

Subjects. The Leiden 85-plus study is a population-based study in which all inhabitants of Leiden, The Netherlands, aged ≥85 years were invited to take part (30). Of a total of 1,258 eligible subjects, 221 died before enrollment, which lasted from December 1, 1986 to March 1, 1988. Of the 1,037 remaining subjects, 977 (94%) participated and were medically interviewed at home. Diabetes was diagnosed on the basis of the previous history or a glucose level >11.0 mmol/liter in a nonfasting blood sample, or the use of medication for treatment of diabetes. After the exclusion of subjects with a non-Dutch (n = 29) or unknown (n = 69) place of birth, sufficient cell material was available from 666 (188 men and 478 women) subjects for the present genetic study. Deoxyribonucleic acid was extracted by protein precipitation using potassium acetate and chloroform. The ACE genotypes were determined as previously described (32). Because the ACE I/D genotype is erroneously mistyped as ACE D/D in up to 5% of cases, the presence of the ACE D/D genotype was confirmed with an insertion-specific polymerase chain reaction (PCR) amplification (33). The PAI-1 4G/5G genotypes were determined using an allele-specific PCR amplification described by Falk et al. (34). It was not possible to determine the ACE genotype in 26 cases and the PAI-1 genotype in 22 cases for technical reasons. The ACE and PAI genotypes were independently assessed by two observers. The study was approved by the Medical Ethics Committee of the Leiden University, and informed consent was obtained from all participants.

Cross-sectional analysis. The ACE and PAI-1 genotype distributions were compared between elderly subjects ≥85 years old and young control subjects. To avoid false associations with the ACE and PAI-1 polymorphisms due to differences in geographic origin rather than age, only those subjects ≥85 years old who were born in Leiden (n = 356, 55%) were compared with a control population that consisted of 250 (139 men and 111 women) blood donors 18 to 40 years old of Dutch descent with either two Leiden-born parents or one Leiden-born parent and the other born within a 12-km distance from Leiden. Information regarding the birthplace of their grandparents was obtained from a written questionnaire.

The elderly subjects were survivors of a cohort born in

Abbreviations and Acronyms
- ACE = angiotensin I–converting enzyme
- CI = confidence interval
- I/D = insertion/deletion
- PAI-1 = plasminogen activator inhibitor-1
- PCR = polymerase chain reaction
- RR = relative risk

Cross-sectional analysis. The ACE and PAI-1 genotype distributions were compared between elderly subjects ≥85 years old and young control subjects. To avoid false associations with the ACE and PAI-1 polymorphisms due to differences in geographic origin rather than age, only those subjects ≥85 years old who were born in Leiden (n = 356, 55%) were compared with a control population that consisted of 250 (139 men and 111 women) blood donors 18 to 40 years old of Dutch descent with either two Leiden-born parents or one Leiden-born parent and the other born within a 12-km distance from Leiden. Information regarding the birthplace of their grandparents was obtained from a written questionnaire.

The elderly subjects were survivors of a cohort born in
Leiden between 1887 and 1901. Therefore, an investigation was made of whether the young control population was likely to represent the Leiden genotype distribution of two generations before. The frequencies of the ACE D/D genotype were 27.1%, 27.0%, 27.5% and 22.4% among young subjects with either one or more (n = 203), two or more (n = 178), three or more (n = 120) and four (n = 76) Leiden-born grandparents, respectively. For the PAI-1 4G/4G genotype these frequencies were 29.6%, 30.9%, 30.8% and 27.6%, respectively. Thus, the ACE and PAI-1 genotype frequencies in control subjects were independent of the number of grandparents born in Leiden, except for the relatively low frequency of the ACE D/D genotype in the young subjects with four Leiden-born grandparents. Among elderly subjects born in Leiden, however, the prevalence of the ACE D/D genotype was somewhat higher (29.2%) as compared with elderly subjects born elsewhere in The Netherlands (28.6%). These data indicate that no specific Leiden ACE or PAI-1 genotype distribution existed around the year 1900.

Prospective follow-up study. All participants in the Leiden 85-plus study were followed up for mortality until October 1, 1996. Among 640 subjects genotyped for the ACE polymorphism and 646 for the PAI-1 polymorphism, two were lost to follow-up, leaving 638 and 644 subjects, respectively, for the prospective follow-up study. Primary causes of death were obtained from the Dutch Central Bureau of Statistics and categorized according to the 9th International Classification of Diseases (ICD-9) (35) for cardiovascular disease (codes 390–459), ischemic heart disease (codes 410–414) and cerebrovascular disease (codes 430–438).

Statistical analysis. In the cross-sectional analysis, distributions of alleles and genotypes were compared by using the chi-square test. Mortality risks up to the age of 85 years were estimated with the exposure odds ratio. In the prospective follow-up study, survival times for subjects were computed from the date of the home visit to the date of one of the following events: death from a specific cause, death from any cause or October 1, 1996. Survival was estimated using the Kaplan-Meier product-limit method. Age- and gender-adjusted (when applicable) mortality risks and 95% confidence intervals (CIs) were estimated with Cox proportional hazards models. Causes of death were assumed to be independent. P values < 0.05 were considered to indicate statistical significance, and all p values were based on two-sided tests. The analyses were performed using the SPSS statistical software, version 6.1 (Chicago, Illinois).

RESULTS

ACE I/D polymorphism. The ACE genotype distribution was 22.5% (I/I), 49.7% (I/D) and 27.8% (D/D) in the cohort of subjects ≥85 years old (n = 640). For the cross-sectional analysis, ACE genotype frequencies in the elderly subjects born in Leiden (n = 353, 55% of the complete cohort) were compared with those in young subjects 18 to 40 years old whose families originated from the Leiden area (n = 250) (Table 1). Genotype frequencies in both groups were in agreement with the distribution predicted by the Hardy-Weinberg equilibrium and similar to that in other white groups (36). The distribution of ACE genotypes was similar in elderly and young subjects (p = 0.46) and also not significantly different for men (p = 0.81) and women (p = 0.81) when analyzed separately. On the basis of genotype frequencies, the mortality risk up to the age of 85 years associated with the ACE D/D genotype was estimated at 0.8 (95% CI 0.5 to 1.2) as compared with that associated with the ACE I/I genotype.

Ten-year survival of the complete cohort of elderly subjects according to ACE genotype is shown in Figure 1. During follow-up, the all-cause mortality rate was 89% and the cardiovascular mortality rate was 38%. The all-cause mortality risk of elderly ACE D/D carriers was 0.9 (95% CI 0.7 to 1.1) as compared with that of ACE I/I carriers (Table 2). The ACE D/D genotype was not associated with the risk of death due to cardiovascular disease (relative risk [RR] 0.8, 95% CI 0.6 to 1.2) (Table 2). Similar estimates were obtained for men and women, except for the ACE I/D genotype. In men, the heterozygous genotype was associated with an increased mortality risk. This is not compatible with a recessive or (co-)dominant effect.

PAI-1 4G/5G polymorphism. The PAI-1 genotype frequencies were 19.2% (5G/5G), 52.9% (4G/5G) and 27.9% (4G/4G) in the cohort of elderly subjects ≥85 years old (n = 646). The PAI-1 genotype distributions in the elderly subjects born in Leiden (n = 354) and in young subjects...
whose families originated from the Leiden area (n = 250) were in Hardy-Weinberg equilibrium (Table 3). The genotype frequencies in young subjects were similar to those previously reported for white subjects (23,25). No overall significant differences were observed in PAI-1 genotype distribution (p = 0.37) or for men and women separately (p = 0.11 and p = 0.78, respectively). The mortality risk up to the age of 85 years associated with the PAI-1 4G/4G genotype was estimated at 0.8 (95% CI 0.5 to 1.2) as compared with that associated with the PAI-1 5G/5G genotype.

Table 2. Ten-Year All-Cause and Cardiovascular Disease Mortality Risks According to Angiotensin-Converting Enzyme Inhibitor Genotype in Subjects ≥85 Years Old

<table>
<thead>
<tr>
<th>ACE Genotype</th>
<th>All-Cause</th>
<th>Cardiovascular Disease</th>
<th>Ischemic Heart Disease</th>
<th>Cerebrovascular Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR*</td>
<td>95% CI</td>
<td>RR</td>
<td>95% CI</td>
</tr>
<tr>
<td>All subjects</td>
<td>n = 569</td>
<td></td>
<td>n = 241</td>
<td></td>
</tr>
<tr>
<td>I/I</td>
<td>1</td>
<td>(0.9–1.4)</td>
<td>1</td>
<td>(0.9–1.8)</td>
</tr>
<tr>
<td>I/D</td>
<td>1.2</td>
<td>(0.7–1.1)</td>
<td>0.8</td>
<td>(0.6–1.2)</td>
</tr>
<tr>
<td>D/D</td>
<td>316</td>
<td></td>
<td>178</td>
<td></td>
</tr>
<tr>
<td>Men only</td>
<td>n = 160</td>
<td></td>
<td>n = 62</td>
<td></td>
</tr>
<tr>
<td>I/I</td>
<td>1.2</td>
<td>(1.1–2.3)</td>
<td>1.7</td>
<td>(0.9–3.3)</td>
</tr>
<tr>
<td>I/D</td>
<td>1</td>
<td>(0.8–1.9)</td>
<td>1.2</td>
<td>(0.6–2.5)</td>
</tr>
<tr>
<td>D/D</td>
<td>51</td>
<td></td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Women only</td>
<td>n = 409</td>
<td></td>
<td>n = 179</td>
<td></td>
</tr>
<tr>
<td>I/I</td>
<td>1</td>
<td>(0.8–1.3)</td>
<td>1</td>
<td>(0.8–1.7)</td>
</tr>
<tr>
<td>I/D</td>
<td>1.2</td>
<td>(0.6–1.0)</td>
<td>0.7</td>
<td>(0.5–1.1)</td>
</tr>
<tr>
<td>D/D</td>
<td>231</td>
<td></td>
<td>127</td>
<td></td>
</tr>
</tbody>
</table>

*Relative risk (RR) indicates the mortality risk as estimated by a Cox proportional hazard model adjusted for age at baseline.
CI = confidence interval; other abbreviations as in Table 1.

Table 3. Plasminogen Activator Inhibitor-1 Genotype Distributions in Subjects ≥85 Years Old and Young Subjects Whose Families Originated From the Same Geographic Region

<table>
<thead>
<tr>
<th>PAI-1 Genotype</th>
<th>Elderly*</th>
<th>Young†</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td>n = 354</td>
<td>n = 250</td>
</tr>
<tr>
<td>5G/5G</td>
<td>67 (18.9%)</td>
<td>59 (23.6%)</td>
</tr>
<tr>
<td>4G/5G</td>
<td>190 (53.7%)</td>
<td>125 (50.0%)</td>
</tr>
<tr>
<td>4G/4G</td>
<td>97 (27.4%)</td>
<td>66 (26.4%)</td>
</tr>
<tr>
<td>Men only</td>
<td>n = 111</td>
<td>n = 139</td>
</tr>
<tr>
<td>5G/5G</td>
<td>17 (15.3%)</td>
<td>36 (25.9%)</td>
</tr>
<tr>
<td>4G/5G</td>
<td>61 (55.0%)</td>
<td>70 (50.4%)</td>
</tr>
<tr>
<td>4G/4G</td>
<td>33 (29.7%)</td>
<td>33 (23.7%)</td>
</tr>
<tr>
<td>Women only</td>
<td>n = 243</td>
<td>n = 111</td>
</tr>
<tr>
<td>5G/5G</td>
<td>50 (20.6%)</td>
<td>23 (20.7%)</td>
</tr>
<tr>
<td>4G/5G</td>
<td>129 (53.1%)</td>
<td>55 (49.5%)</td>
</tr>
<tr>
<td>4G/4G</td>
<td>64 (26.3%)</td>
<td>33 (29.7%)</td>
</tr>
</tbody>
</table>

*Median age 89 years (range 85 to 100). †Median age 31 years (range 18 to 40). Data are presented as the number (%) of subjects.
PAI-1 = plasminogen activator inhibitor-1.
The analyses were repeated for elderly subjects with diabetes (n = 69) because they can be expected to have elevated plasma triglycerides, insulin and glucose. In this subset, the all-cause mortality risks associated with the PAI-1 4G/4G and 4G/5G genotypes were 0.4 (95% CI 0.2 to 0.9) and 0.6 (95% CI 0.3 to 1.2), respectively, as compared with that associated with the 5G/5G genotype.

**Combined effects of ACE I/D and PAI-1 4G/5G polymorphisms.** The prevalence of homozygosity for both the ACE and the PAI-1 risk allele was similar among elderly subjects and young subjects of Leiden origin (6.3% and 7.6%, respectively; data not shown). Separate analysis of men and women did not reveal significant differences.

The 10-year mortality risk of elderly subjects carrying both the ACE D/D and PAI-1 4G/4G genotype was 0.8 (95% CI 0.6 to 1.2) from any cause and 0.9 (95% CI 0.5 to 1.5) from cardiovascular disease as compared with that of a reference group of double heterozygous subjects and subjects homozygous for the ACE I-allele and/or the PAI-1 5G-allele. It was not possible to test for an association with death from ischemic heart disease because the number of subjects was too small.

**DISCUSSION**

ACE I/D polymorphism. No evidence was obtained for increased (cardiovascular) mortality among carriers of the ACE D/D genotype in the cross-sectional comparison of young and elderly subjects or in the 10-year follow-up study among elderly subjects. Other cross-sectional studies also showed similar ACE D/D frequencies in elderly subjects of about the same age as those in our study (11,37). A decreased prevalence of the ACE D/D genotype in hypertensive subjects ≥60 years old was reported, suggesting that the ACE D/D genotype may confer an increased mortality in this high risk subgroup (38). Increased mortality in the population at large has, however, not been observed in our study.

Although the neutral ACE I/D polymorphism explains 14% to 25% of the variance in ACE level (1,2), there is much debate about the significance of the relation between the ACE polymorphism and cardiovascular disease. The conflicting results of the studies performed thus far can be attributed to differences in clinical phenotype (such as myocardial infarction and coronary artery disease), differences in the age of the subjects, representativeness of control subjects and differences in environmental and genetic background. Moreover, a meta-analysis of the association between the ACE I/D polymorphism and myocardial infarct-

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**Table 4. Ten-Year All-Cause and Cardiovascular Disease Mortality Risks According to Plasminogen Activator Inhibitor-1 Genotype in Subjects ≥85 Years Old**

<table>
<thead>
<tr>
<th>PAI-1 Genotype</th>
<th>All-Cause</th>
<th>Cardiovascular Disease</th>
<th>Ischemic Heart Disease</th>
<th>Cerebrovascular Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR* 95% CI</td>
<td>RR 95% CI</td>
<td>RR 95% CI</td>
<td>RR 95% CI</td>
</tr>
<tr>
<td>All subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5G/5G</td>
<td>1 n = 575</td>
<td>1 n = 242</td>
<td>1 n = 61</td>
<td>1 n = 78</td>
</tr>
<tr>
<td>4G/5G</td>
<td>0.8 (0.7–1.0) n = 340</td>
<td>0.9 (0.6–1.3) n = 180</td>
<td>0.9 (0.5–2.0) n = 61</td>
<td>0.7 (0.4–1.4) n = 19</td>
</tr>
<tr>
<td>4G/4G</td>
<td>0.9 (0.7–1.1) n = 180</td>
<td>1.6 (0.8–3.3) n = 180</td>
<td>0.7 (0.4–1.4) n = 180</td>
<td></td>
</tr>
<tr>
<td>Men only</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5G/5G</td>
<td>1 n = 159</td>
<td>1 n = 61</td>
<td>1 n = 19</td>
<td>1 n = 19</td>
</tr>
<tr>
<td>4G/5G</td>
<td>1.9 (0.8–1.8) n = 95</td>
<td>1.3 (0.6–2.6) n = 45</td>
<td>3.3 (0.4–27) n = 95</td>
<td>2.5 (0.6–11) n = 45</td>
</tr>
<tr>
<td>4G/4G</td>
<td>1.2 (0.8–2.0) n = 54</td>
<td>1.6 (0.7–3.4) n = 54</td>
<td>8.2 (1.0–64) n = 54</td>
<td>1.9 (0.4–10) n = 54</td>
</tr>
<tr>
<td>Women only</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5G/5G</td>
<td>1 n = 416</td>
<td>1 n = 181</td>
<td>1 n = 42</td>
<td>1 n = 59</td>
</tr>
<tr>
<td>4G/5G</td>
<td>0.7 (0.6–0.9) n = 245</td>
<td>0.8 (0.5–1.1) n = 45</td>
<td>0.7 (0.3–1.5) n = 45</td>
<td>0.7 (0.4–1.3) n = 45</td>
</tr>
<tr>
<td>4G/4G</td>
<td>0.8 (0.6–1.0) n = 126</td>
<td>0.7 (0.5–1.1) n = 61</td>
<td>0.9 (0.4–2.2) n = 126</td>
<td>0.6 (0.3–1.2) n = 126</td>
</tr>
</tbody>
</table>

*Relative risk (RR) indicates the mortality risk as estimated by a Cox proportional hazard model adjusted for age at baseline.
CI = confidence interval; PAI-1 = plasminogen activator inhibitor-1.
A number of relatively large studies reported an association of the ACE D/D genotype with a history of myocardial infarction (4–7). In some studies the association was found to be stronger (4,39) or present only (9,15) in subgroups otherwise at a low risk using different criteria. Furthermore, the prevalence of the ACE D/D genotype was increased among patients who had a fatal myocardial infarction. However, most larger studies, eight in total including the only prospective study, found no evidence for an association with myocardial infarction (8–14) or ischemic heart disease (15). Also, in most studies, no association was observed in low risk subjects (5,8,10,12,13). Our observation—that the ACE D/D genotype was not associated with mortality or fatal cardiovascular disease in old age—indicates that the overall effect of the ACE polymorphisms on fatal disease is probably limited.

Schächter et al. (16) reported an increased prevalence of the ACE D/D genotype in French centenarians as compared with subjects 20 to 70 years old. They suggested that the ACE D/D genotype might be deleterious in middle age, but beneficial to survival in very old age. Recently, the same group developed a mathematic model predicting trajectories of genotype frequencies in aging cohorts as a consequence of differential population mortality rates for the genotypes (41). This model indicated that any genotype with an effect on survival before 95 years of any exhibits an opposite effect after this age. The increased frequency of the ACE D/D genotype in French centenarians was thus explained by assuming an increased mortality of ACE D/D carriers before 95 years old. Neither in our cross-sectional analysis nor in the prospective follow-up study among subjects ≥85-years-old could we find support for this assumption. In addition, no increased ACE D/D frequency was found in 187 Danish centenarians (42).

**PAI-1 4G/5G polymorphism.** We found that men ≥85 years old carrying the PAI-1 4G/4G genotype were at a threefold increased risk of death due to ischemic heart disease during a 10-year follow-up period. This was not reflected in the risk of death from all cardiovascular causes combined or increased mortality before the age of 85 years. The indication that the PAI-1 polymorphism may contribute to the development of ischemic heart disease in old age significantly extends the previous findings that the PAI-1 4G/4G genotype is a risk factor for premature myocardial infarction, which has a much lower incidence (patients <45 years old [18], mean age 58 years [24,26]). It should, however, be noted that although our study is prospective and thus not prone to bias, our findings are based on the analysis of a small subset and therefore require confirmation in more extensive studies.

In contrast to our studies and those mentioned earlier, as well as a study showing a higher prevalence of the PAI-1 4G/4G genotype among subjects with a family history of coronary heart disease (25), the PAI-1 polymorphism was not associated with myocardial infarction in the Etude Cas-Temoins sur l’Infarctus du Myocardie (ECTIM) study (men 25 to 64 years old) (19) and the Physicians’ Health Study (men 40 to 84 years old, mean follow-up eight years) (23). The variable outcomes of these studies may have resulted from chance, but may also point to population differences with respect to the pathogenesis of myocardial infarction or the prevalence of environmental factors modifying the effect of the PAI-1 polymorphism.

The current study could not provide an explanation for the gender-specificity of the association with fatal ischemic heart disease. One previous study also reported data on both genders separately, and these showed that the PAI-1 4G/4G genotype was a risk factor for myocardial infarction in men only (26). Hormonal differences with respect to estrogen are not likely to play a role because the elderly women were well beyond their menopause. Smoking has been suggested to modify the effect of the polymorphism (43). Different smoking habits between men and women might have explained our observation, but the numbers were too small to test for this possibility. Another explanation could have been a different prevalence of diabetes in men and women, because the effect of the PAI-1 4G/4G genotype is known to be more pronounced in individuals with features of insulin resistance (elevated levels of triglycerides, insulin and glucose) (20,21). However, none of the men who carried the apparently deleterious PAI-1 genotype and died of ischemic heart disease had been diagnosed as having diabetes. Overall, the (cardiovascular) mortality was not increased in elderly subjects carrying the 4G/4G genotype with diabetes. In contrast, the mortality risk in these patients was even significantly reduced. We do not have an explanation for this finding.

**Interaction.** Recent studies showed that the PAI-1 4G/4G as well as the ACE D/D genotype is associated with increased PAI-1 levels (28,29). Although the possible deleterious effect on mortality of a decreased fibrinolytic capacity might have been more readily observed in subjects carrying both risk genotypes, none of our analyses indicated an increased mortality in this subgroup.

**Study limitations.** Our study indicates that it is unlikely that the putative ACE and PAI-1 risk genotypes have a major influence on mortality in the population at large. On the basis of the 95% CIs, the overall mortality risks can be expected to be <1.2-fold increased. The current study does not, however, have sufficient power to exclude associations between polymorphisms and specific causes of death, owing to the relatively small numbers of subjects in these subsets. In these subsets, nondifferential misclassification of causes of death could be expected to have occurred because they were not confirmed at necropsy, which leads to an underestimation of the risk estimates. We have previously reported associations with single specific causes of death, indicating that the registry data have significant power to
discriminate between the various causes of death (30,31,44). Furthermore, it has been shown in the United Kingdom that underreporting of cerebrovascular and cardiovascular mortality as an underlying cause of death occurs relatively infrequently (45). In the case of >90% of the individuals who died within four weeks of hospital admission because of stroke or ischemic heart disease, the disease was mentioned on the death certificate.

Conclusions. We conclude that the influence on disease risk of moderately increased ACE and PAI-1 activities that are associated with the ACE D/D and the PAI-1 4G/4G genotype is not large enough to affect mortality in the general population. Our data suggest, however, that the PAI-1 4G/4G genotype may play a role in fatal ischemic heart disease in elderly men. This observation requires confirmation in larger prospective studies.

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