Breast cancer presents a serious public health risk in both developed and developing countries. With 1 million new cases diagnosed in the world annually, it accounts for 18% of all female malignancies (1, 2). Risk factors for this disease vary from lifestyle to genetic factors (3), which are estimated to account for between 15% to 25% of the cases (4). Germline mutations in high-penetration genes, such as \( \text{BRCA}_1 \) and \( \text{BRCA}_2 \), explain 5% of all breast cancer cases (4). Most likely, the genetic susceptibility to breast cancer is explained by multiple highly penetrant and many lower-penetrance mutations (5). The genes involved in breast cancer are expected to be responsible for key processes in cell growth regulation and cell proliferation, including angiogenesis (6). One of the newly studied angiotensinogen and growth factors is angiotensin II (7), which has a wide spectrum of target tissues, including breast epithelial cells. It has a variety of functions, acting as a growth factor both in normal and cancer epithelial breast cells and promoting angiogenesis (7, 8). Angiotensin II is converted from angiotensinogen I by the angiotensin-converting enzyme (ACE). Studies conducted to assess the role of ACE and ACE inhibitors in both breast cancer and cancer in general show contradicting results. Whereas ACE inhibitors have been shown to block the processes of angiogenesis and tumor growth both in vivo and in vitro (9, 10), findings on the protective effect of ACE inhibitors on cancer still remain inconsistent. Whereas Lever et al. (11) found a decreased risk of cancer in patients taking ACE inhibitors, Li et al. (12) and Friis et al. (13) showed no protective effect of these drugs. An alternative way to study the role of ACE in cancer is to study the gene encoding for this enzyme. The ACE gene, which is located in chromosome 17q23, has many polymorphisms. The most commonly studied is a 287 bp \( \text{Alu} \) insertion/deletion (I/D) polymorphism in intron 16 that accounts for 50% of the variability in circulating ACE levels (14-16) and has been shown to be in complete linkage disequilibrium with the putative ACE-linked quantitative trait locus in Caucasians (15, 16). Furthermore, Koh et al. (17) showed that Chinese women who carried the I allele of the ACE I/D polymorphism had a lower risk of developing breast cancer.

In this study, we evaluated the relationship of the I/D polymorphism in the ACE gene to breast cancer risk in a population-based study of Caucasian postmenopausal women.

**Patients and Methods**

**Study Population.** Our study is part of the Rotterdam Study, a population-based follow-up study of determinants of diseases in the elderly. All inhabitants of Ommoord, a suburb of Rotterdam, ages 55 years or older were invited to participate, of whom 7,983 agreed (78.1%). The design of the study has been previously described (18). Informed consent was obtained from all subjects, and the Medical Ethics Committee of the Erasmus Medical Center approved the study. The study population consisted of all 4,878 female postmenopausal participants.
in situ diagnoses until February 2003, both mammography was implemented in 1991 for women ages 50 to 69 and cytopathology. Furthermore, a biannual screening mammography was obtained from the discharge registries of all hospitals in the Netherlands. Confirmed breast cancer diagnoses and incidence dates were obtained from the discharge registries of all hospitals in Rotterdam, the Daniel den Hoed cancer clinic, and Pathological Anatomical District Automatized Archives (21), a Dutch nationwide network and registry of histopathology and cytopathology. Furthermore, a biannual screening mammography was implemented in 1991 for women ages 50 to 69 years and since 1998 also for women 70 to 74 years (22). All diagnoses until February 2003, both in situ and invasive carcinomas, were included in the analyses.

Genotyping. The ACE I/D polymorphism was genotyped in 4,117 (84.4%) of the women in the Rotterdam Study (84.4%). DNA was isolated from blood samples using standard procedures (salting out method; ref. 23). The II, ID, and DD genotypes were detected by using the PCR technique according to the method of Lindpainter et al. (24) with modifications. The genotype procedure has been already described (25).

Data Analysis. Hardy-Weinberg equilibrium of the I/D polymorphism was tested using Markov-Chain Monte-Carlo approximation of the exact test, as implemented in the GENEPOP package V 3.3 (26). Categorical variables (parity, hormone replacement therapy, smoking, antihypertensive drug use, and ACE inhibitors use) were compared between genotype groups using the chi-squared test. Continuous variables, which were not normally distributed (age at entry, age at menopause, and BMI), were compared using the independent-sample Mann-Whitney test. We conducted the analysis in two steps. First, we used logistic regression to study the risk of breast cancer by ACE genotype. We adjusted for possible confounders, such as age at entry and age at menopause, and we stratified for parity, hormone replacement therapy, smoking, antihypertensive drug use, and BMI, generating five models. Second, and to calculate disease-free survival by ACE genotype, a Cox proportional hazards model was fitted using age as the underlying time of the model and taking the II genotype as the reference category. Further stratification was done by parity, hormone replacement therapy, and BMI to study interactions between the gene and other risk factors associated with breast cancer. The covariates used in both analyses were used because of their well-documented importance as risk factors for breast cancer (11, 12, 27-29) or their association with genotype (20, 25). Logistic regression analysis was performed using SPSS for Windows software package version 11.0 and the survival analyses were carried out using the S-plus program version 6.

Results

Of a total of 4,878 postmenopausal women included in our study, 4,117 (84.4%) were successfully genotyped. Of these women, 8.1% were lost to follow-up. Loss of follow-up was not associated with ACE genotype or to risk factors for breast cancer. The frequencies of the I/D genotypes of the ACE gene were in Hardy-Weinberg equilibrium proportions ($P = 0.96$). The distribution of the studied variables was not significantly different between genotypes (Table 1). The distribution of ACE genotypes (data not shown).

There were 87 (2.1%) women who entered the study with previously diagnosed breast cancer and 114 (3.4%) were diagnosed during follow-up. The prevalent cases were excluded from all analyses. The number of breast cancer cases by genotype is shown in Fig. 1. The figure shows that the number of breast cancer patients increases as the number of D alleles increases ($P_{trend} = 0.02$).

The logistic regression yielded an odds ratio (OR) of 1.86 [95% confidence interval (95% CI), 1.06-3.27; $P = 0.03$] for DD carriers. Further adjustment of this model for hormone replacement therapy produced the same results. Adjustment for antihypertensive drug use provided an OR of 1.90 (95% CI, 1.06-3.27; $P = 0.3$) for DD versus II carriers. This association remained significant when additionally adjusting for parity (OR, 1.79; 95% CI, 1.06-3.27; $P = 0.03$), smoking (OR, 1.83; 95% CI, 1.04-3.21; $P = 0.03$), and BMI (OR, 2.06; 95% CI, 1.14-3.71; $P = 0.02$).

Table 1. General characteristics of the study population stratified by ACE I/D genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>DD</th>
<th>ID</th>
<th>II</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total studied (%)</td>
<td>1,170 (28.4%)</td>
<td>2,247 (49.7%)</td>
<td>900 (21.9%)</td>
<td>4,117</td>
</tr>
<tr>
<td>Mean age of entry (SD)</td>
<td>70.49 (9.82)</td>
<td>70.48 (9.54)</td>
<td>69.48 (9.80)</td>
<td>71.65 (10.27)</td>
</tr>
<tr>
<td>Mean age at death</td>
<td>83.93 (9.41)</td>
<td>84.45 (8.51)</td>
<td>84.05 (8.62)</td>
<td>84.20 (8.81)</td>
</tr>
<tr>
<td>Mean age at menopause (SD)</td>
<td>48.76 (5.23)</td>
<td>48.9 (5.04)</td>
<td>48.72 (5.27)</td>
<td>48.88 (5.2)</td>
</tr>
<tr>
<td>Mean no. children</td>
<td>2.06 (1.73)</td>
<td>2.09 (1.69)</td>
<td>2.17 (1.76)</td>
<td>2.10 (1.71)</td>
</tr>
<tr>
<td>Parity (%; ≥1 child)</td>
<td>885 (76.3%)</td>
<td>1,562 (79.3%)</td>
<td>683 (79.3%)</td>
<td>641.7 (76.03%)</td>
</tr>
<tr>
<td>Presence of concession therapy (%)</td>
<td>126 (10.8%)</td>
<td>220 (10.7%)</td>
<td>105 (11.7%)</td>
<td>451 (10.95%)</td>
</tr>
<tr>
<td>Use of antihypertensives</td>
<td>160 (13.9%)</td>
<td>257 (12.8%)</td>
<td>98 (11.1%)</td>
<td>515 (12.8%)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>430 (36.8%)</td>
<td>752 (36.7%)</td>
<td>299 (33.2%)</td>
<td>1,481 (35.97%)</td>
</tr>
<tr>
<td>Mean BMI (SD)</td>
<td>26.79 (4.12)</td>
<td>26.67 (4.12)</td>
<td>26.72 (4.02)</td>
<td>26.71 (4.09)</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>181 (15.5%)</td>
<td>351 (17.1%)</td>
<td>171 (19%)</td>
<td>433 (10.52%)</td>
</tr>
</tbody>
</table>

NOTE: All $P$ values ≥ 0.05.

Measurements. At baseline, information concerning age, smoking behavior, parity and number of children, hormone replacement therapy, age at menopause, and medical history was obtained by an interview (18). Body mass index (BMI) was calculated by dividing the weight in kilograms by the height (in meters) squared (19). Blood pressure measurement has been previously described (20).

Cancer Diagnosis. General practitioners reported the cases through a computer system covering 80% of the study population. For those participants not covered, research physicians visit general practitioners to record all morbidity. Finally, to acquire a complete ascertainment, histologically confirmed breast cancer diagnoses and incidence dates were obtained from the discharge registries of all hospitals in Rotterdam, the Daniel den Hoed cancer clinic, and Pathological Anatomical District Automatized Archives (21), a Dutch nationwide network and registry of histopathology and cytopathology. Furthermore, a biannual screening mammography was implemented in 1991 for women ages 50 to 69 years and since 1998 also for women 70 to 74 years (22). All diagnoses until February 2003, both in situ and invasive carcinomas, were included in the analyses.

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We conducted an association study to evaluate the relationship between breast cancer–free survival by ACE I/D markers will yield little extra information. Strong linkage disequilibrium implies that testing additional functional ones in this gene, as measured as the relation to variability of plasma ACE levels (39). Furthermore, this population, this polymorphism explains around 28% of the 16. It has been previously reported that in a subset of our gene. Here, we only tested the ACE I/D polymorphism in intro patients included both premenopausal and postmenopausal large enough to evaluate ethnic specific risks, and their reduction risk of breast cancer independently of environmental and other familial risk factors for the disease. On the other hand, Haiman et al. (17) conducted a study among Chinese postmenopausal women in which they found that individuals carrying the II genotype had a significantly reduced risk of breast cancer independently of environmental and other familial risk factors for the disease. The other hand, Haiman et al. (38) did a case-control study in a multiethnic cohort where they observed a modest positive association between the II genotype and breast cancer risk in African Americans. They did not, however, see the association consistently in all ethnic groups. Furthermore, although they had a large sample within each ethnic group, it was not large enough to evaluate ethnic specific risks, and their patients included both premenopausal and postmenopausal women. A large number of polymorphisms are known in the ACE gene. Here, we only tested the ACE I/D polymorphism in intro 16. It has been previously reported that in a subset of our population, this polymorphism explains around 28% of the variability of plasma ACE levels (39). Furthermore, this polymorphism is in strong linkage disequilibrium with the functional ones in this gene, as measured as the relation to ACE levels or cardiovascular disease outcomes (15, 16). The strong linkage disequilibrium implies that testing additional markers will yield little extra information. Thus far, and to our knowledge, no follow-up study has been done to assess breast cancer–free survival by ACE I/D Table 2. Hazard ratios for breast cancer by genotype

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>II</th>
<th>ID</th>
<th>DD</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>3,724</td>
<td>1.23</td>
<td>1.80</td>
<td>1.07-3.01*</td>
</tr>
<tr>
<td>No use of hormone replacement therapy</td>
<td>3,290</td>
<td>1.60</td>
<td>2.13</td>
<td>1.18-3.86*</td>
</tr>
<tr>
<td>Use hormone replacement therapy</td>
<td>444</td>
<td>0.25</td>
<td>0.79</td>
<td>0.26-2.42</td>
</tr>
</tbody>
</table>

*P < 0.05.

Discussion

We conducted an association study to evaluate the relationship between the ACE I/D polymorphism and the risk of breast cancer, and we did so in two steps. Our analysis showed that DD carriers have an increased risk of developing breast cancer. When analyzing this group, all further adjusted models showed significantly increased risks for DD carriers when compared with II carriers. We also report a linear increase of breast cancer risk with the presence of the D allele of I/D polymorphism in the ACE gene. For premenopausal women, the BRCA 1 and BRCA 2 genes have been associated with an increased risk for breast cancer (5, 27, 30-32). A vast literature suggests that variants in genes that regulate cell growth are involved in the development of this disease (5, 33). Moreover, several studies have shown that angiotensin II acts as a growth factor in normal and breast cancer cells through phospholipase C activation (8, 34-37). Koh et al. (17) conducted a study among Chinese postmenopausal women in which they found that individuals carrying the II genotype had a significantly reduced risk of breast cancer independently of all our proposed known risk factors. Hormone replacement therapy and parity did not weaken our association between the I/D polymorphism and breast cancer risk. Our results suggest that the ACE I/D polymorphism may play an important role as susceptibility factor in breast cancer risk and disease-free survival in Caucasian postmenopausal women.

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References

Angiotensin-Converting Enzyme Gene Insertion/Deletion Polymorphism and Breast Cancer Risk


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