Angiotensin I-Converting Enzyme (ACE) Gene Polymorphism and Breast Cancer Risk among Chinese Women in Singapore

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ABSTRACT

Angiotensin II has been shown to have possible mitogenic and angio-
genic effects in human cell lines and animal models of breast cancer. It is converted from its precursor to its active form by the angiotensin I-
 converting enzyme (ACE). A recent epidemiological study observed lower breast cancer incidence in female users of ACE inhibitors relative to
nonusers with comparable cardiovascular conditions. To study the hy-
pothesis that reduced ACE activity is associated with reduced risk of
breast cancer, we conducted a nested case-control study within the Sin-
apore Chinese Health Study Cohort to investigate the associations be-
tween the ACE A-240T and I/D gene polymorphisms, and breast cancer
risk. For this analysis, 189 incident breast cancer cases and 671 female
cohort control subjects were compared. The low-activity A and I alleles
were the putative “low-risk” alleles. The I/D and A-240T genotypes ex-
hibited significant linkage disequilibrium among Singapore Chinese (con-
tingency coefficient = 0.74; P < 0.001). With adjustment for breast cancer
risk factors, women with one or two copies of the low activity A allele
showed a statistically significant reduction in risk compared with those
with the TT genotype [odds ratio (OR), 0.55; 95% confidence interval (CI),
0.34–0.90]. The risk reduction was enhanced after excluding subjects with
medical conditions for which ACE inhibitors are commonly prescribed
(OR, 0.49; 95% CI, 0.27–0.89). Comparable results were obtained with
respect to the I/D genotype and risk of breast cancer. When the I/D and
A-240T genotypes were considered simultaneously, compared with women
with the high-activity genotypes (either TT or DD or both), those with the
low-activity genotypes (presence of both A and I alleles) exhibited lower
breast cancer risk (OR, 0.46; 95% CI, 0.27–0.81). Our findings support
experimental data implicating ACE and angiotensin II in breast cancer,
and suggest that the renin-angiotensin system may serve as a therapeutic
target for breast cancer treatment and prevention.

INTRODUCTION

Angiotensin II is an aldosterone-stimulating peptide with a direct,
potent vasopressive effect on the peripheral vasculature, and plays a
pivotal role in electrolyte and circulatory homeostasis. It is converted
from its precursor, angiotensin I, by the catalytic action of the dipep-tidyl carboxypeptidase known as ACE,3 which is present as a mem-
brane-bound enzyme on the surface of a variety of cell types as well
as in a secreted form. Plasma ACE levels are highly genetically
determined, and as much as 47% of interindividual variability of
plasma ACE concentration is determined by the presence [insertion
(I)] or absence [deletion (D)] of a 287-bp Alu-type sequence in intron
16 of the ACE gene in the Caucasian population. Homozygotes for the
I allele can display as low as half of the ACE level compared with the
homozygotes for the D allele, whereas the ID heterozygotes display an
intermediate level (1). Another polymorphism that has been described
more recently is the A-240T polymorphism, with the A allele being
associated with a lower ACE level compared with the T allele (2). The
level of plasma ACE has an important influence in the pathophysi-
ology of cardiovascular disease, and polymorphism in the ACE gene
has been shown to influence risk of hypertension (3–5) and other cardiac
disease (6, 7).

Evidence from animal models has suggested that angiotensin II
stimulates neovascularization (8, 9) by promoting arteriolar smooth
muscle cell proliferation (10), and this has led to increased interest in
the role that angiotensin II may play in promoting angiogenesis in
neoplastic growth. In addition, angiotensin II may act as a mitotic
factor by inducing or regulating gene expression in cell cycle pro-
gression (11, 12), and angiotensin II receptor blockade effectively
reduced transforming growth factor β1-dependent tumor progression
in vivo (13). Captopril, a prototype ACE inhibitor, has been shown to
inhibit proliferation in a variety of cell types, including human breast
cancer cells (14–17), and to reduce tumor growth in experimental
models of cancer (18, 19).

ACE inhibitors are used widely as antihypertensive agents, and
several epidemiological studies have examined cancer risks in
long-term users of ACE inhibitors. Lever et al. (20) studied a
cohort of hypertensive patients who received treatment and medi-
cal follow-up at the Glasgow Blood Pressure Clinic. Users of ACE
inhibitors exhibited a reduced risk of cancer when compared with
general population rates based on similarly aged women in West
Scotland (RR = 0.63; 95% CI, 0.41–0.93). This reduction in
cancer risk was most prominent for female breast cancer
(RR = 0.33). However, three subsequent observational studies
(two case-control studies and a population-based cohort study)
failed to confirm the ACE inhibition-reduced breast cancer risk
association noted previously (21–23). A fourth study, a random-
ized, 3-arm trial among elderly hypertensive patients in Sweden,
assigned to ACE inhibitors, calcium antagonists, or diuretics/β-
blockers, also did not find a decreased incidence of breast cancer
in female users of ACE inhibitors compared with either other
female trial participants or general population rates (24).

As an additional step in testing the hypothesis that reduction of
ACE levels protects against cancer, we examined whether function-
ally significant polymorphisms of the ACE gene were related to breast
cancer risk within a population-based, prospective cohort of Chinese
women in Singapore. If down-regulation of ACE protects against
breast cancer risk, our model would predict that individuals possess-
ing the low-activity alleles (i.e., the I and A alleles) are at a reduced
risk of breast cancer relative to those with the DD or TT genotype,
respectively.

MATERIALS AND METHODS

Study Population.

The subjects were participants of the Singapore Chi-
nese Health Study, a population-based, prospective investigation of diet and
cancer risk. From April 1993 through December 1998, a total of 63,257
Chinese women and men 45–74 years of age enrolled in the study (only
women are included in this report; Ref. 25). We restricted study subjects to

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2 The abbreviation used is: ACE, angiotensin I-converting enzyme; RR, relative risk;
CI, confidence interval; OR, odds ratio; BMI, body mass index; I/D, insertion deletion.

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the two major dialect groups of Chinese in Singapore: the Hokkiens who originated from the southern part of Fujian Province, and the Cantonese who came from the central region of Guangdong Province. The subjects were residents of government housing estates; 86% of the Singapore population resided in these facilities. At recruitment, a face-to-face interview was conducted in the home of the subject by a trained interviewer, using a structured questionnaire that requested information on demographics, lifetime use of tobacco, menstrual and reproductive history (women only), medical history, and family history of cancer. The questionnaire also included a dietary component assessing current intake patterns (including questions about coffee, tea, and alcoholic beverages), which was subsequently validated against a series of 24-h diet recalls (25).

Between April 1994 and July 1999, we attempted to collect blood and single-void urine specimens from a random 3% sample of study enrollees. A 20-ml blood sample was obtained from each consenting subject. Immediately after blood collection, the tubes were put on ice during transport from the homes of the subjects to the laboratory. All of the specimens were then separated into their various components (plasma, serum, RBCs, anduffy coat). All of the specimens were subsequently stored in a liquid nitrogen tank at −180°C until August 2001, when they were moved to a −80°C freezer for long-term storage. If the subject refused to donate blood, he/she was asked to donate buccal cells, which were collected through the use of a modified “mouthwash” protocol based on published methods (26, 27). The subject was provided with a new toothbrush and asked to clean their teeth thoroughly. After an interval of 20 min, during which no food or drink was consumed, they were given 10 ml of commercially purchased “Listerine” mouthwash and asked to-swish the liquid vigorously in their mouths for 60 s. The mouthwash was then collected in a sterile 50-ml polypropylene tube, put on ice, and brought back to the laboratory within 5 h, where it was stored at −30°C. Of 1059 female cohort participants contacted for sample donation, blood (n = 514) or buccal cells (n = 164) were collected from 678 subjects, representing a participation rate of 64%. The control group for the present study comprised of the 671 women who remained free of breast cancer as of December 31, 2000.

We identified incident breast cancer cases through the population-based cancer registry in Singapore (28). As of December 31, 2000, 321 cases of incident breast cancer had developed among female cohort subjects. Historical and staging information of all of the breast cancer diagnoses were confirmed by manual review of the pathology reports and clinical charts. Blood (n = 145) or buccal (n = 44) specimens were available on 189 (59%) of the breast cancer cases. Of these 189 cases of breast cancer, 16 cases had in situ cancers, 51 had stage I, 89 had stage II, 17 had stage III, and 15 had stage IV tumors, whereas staging information was unavailable on 1 case. The 121 cases who had stage II or higher tumors (regional and metastatic disease) were classified as having advanced disease. Breast cancer cases who did not give a blood or buccal cell sample were less educated than those who provided such a sample (42% versus 29% had no formal education); the two groups were otherwise similar with respect to age at cancer diagnosis (mean, 60 versus 59 years) and dialect group (42 versus 48% Cantonese).

Informed consent forms were completed by all of the participants at baseline interviews, and time of collection of blood (or buccal cells) and urine specimens. The Institutional Review Boards at the University of Southern California and the National University of Singapore had approved this study.

**Genotyping Methods.** For laboratory analysis, blood and buccal cell samples were shipped on dry ice to the University of Southern California. Genomic DNA was purified from buffy coats of peripheral blood (145 cases and 507 controls) and buccal cell samples (44 cases and 164 controls) using the PureGene Blood kit (Gentra Systems, Minneapolis, MN) or a QIAamp 96 DNA Blood kit (Qiagen, Valencia, CA). Genotyping was performed using the fluorogenic 5’-nucleotide assay (TaqMan Assay; Ref. 29).

The TaqMan assays were performed using a TaqMan PCR Core Reagent kit (Applied Biosystems, Foster City, CA) according to manufacturer’s instructions. Amplification of the region of ACE containing the ID polymorphism was accomplished using a common oligonucleotide for both alleles in the forward direction and a unique oligonucleotide for each allele in the reverse direction. The oligonucleotide primers used for the ACE ID genotyping were GC008f (5’-CCCTTCCCATTTTCCTAAGACTG-3’) and GC008rev1 (5’-GCTCAGAAGATTTCAGACCTGA-3’) for the D allele, and GC008rev2 (5’-GATCCCGCCACTGCACCTC-3’) for the I allele. In addition, the fluorogenic oligonucleotide probes used to detect each of the alleles were GC008F (5’-TGCTTATAGCTAATTTTAGGTGTTCG-3’) labeled with 6-carboxyfluorescein to detect the D allele and GC008C (5’-CTCGCTGTGGGCCCACTC-3’) labeled with CY3 (Biosearch Technologies, Novato, CA) to detect the I allele. PCR amplification using ~10 ng of genomic DNA was performed in a thermal cycler (MWG Biotech, High Point, NC) with an initial step of 95°C for 10 min followed by 50 cycles of 95°C for 25 s and 62°C for 1 min.

The oligonucleotide primers for amplification of the region of the ACE gene containing the A-240T polymorphism were GC015for (5’-TGTTGGCCGAAAATGTCG-3’) and GC015rev (5’-CCGAAGAGGACTCGGAG-3’). The fluorogenic oligonucleotide probes used to detect each of the alleles were GC015F (5’-CTCCCTCTTCTTTGGAGATGGGC-3’) labeled with 6-carboxyfluorescein to detect the A allele and GC015C (5’-CTCCCTCTTTTCTTGAAGATGGGC-3’) labeled with CY3 to detect the T allele. In addition, the probes were synthesized with a modified base (propyne dU) in place of the T residues to increase the overall thulium of the oligonucleotide (Biosearch Technologies). PCR amplification using ~10 ng of genomic DNA was performed in a thermal cycler with an initial step of 95°C for 10 min followed by 50 cycles of 95°C for 25 s and 62°C for 1 min.

After PCR amplification, the fluorescence profile of each well was measured in an ABI 7900HT Sequence Detection System (Applied Biosystems) and the results analyzed with Sequence Detection Software (Applied Biosystems). Experimental samples were compared with 12 controls to identify the three genotypes at each locus. Any samples that were outside the parameters defined by the controls were identified as noninformative and were retested. All of the samples were processed without knowledge of their case/control status. Of the 860 study subjects, 17 subjects had noninformative A-240T genotype only, 28 had noninformative ID genotype only, and 7 had both noninformative A-240T and ID genotypes. Therefore, these subjects were excluded from data analyses related to those genotypes.

**Statistical Analysis.** We examined the association between the ACE ID and A-240T genotypes among all of the study subjects by the contingency coefficient (30).

Although we sampled our controls from the whole cohort, this study is more case-control than case-cohort in design because the time period of follow-up was only comparable between cases and the subcohort, with only 7 subjects in the latter group developing breast cancer during the observation period. Nonetheless, we conducted parallel analyses using standard case-control and case-cohort methods, and the two sets of results were similar. The data presented in the paper were based on the case-control analysis. Specifically, we used unconditional logistic regression methods (31) to examine the effects of the A-240T and ID gene polymorphisms on breast cancer risk. The I allele of the ID gene polymorphism and the A allele of the A-240T gene polymorphism have been found to be associated with reduced ACE levels (1, 2). When we considered the genotypes of both polymorphisms simultaneously, individuals possessing both the I and A alleles were classified as having the “low-activity” genotype, whereas the remaining subjects (i.e., those with either the TT or ID genotypes) were considered as having the “high-activity” genotype. The strength of the gene-cancer association was measured by ORs and their corresponding 95% CIs, and Ps using high-activity genotypes as the reference category. In addition to age at recruitment, year of recruitment, and dialect group (Cantonese or Hokkien), level of education (none, primary, and secondary school or higher) was included as a covariate in all of the regression models. The following known risk factors for breast cancer were also included in regression models when the effect of the ACE genotype on breast cancer risk was examined: age (year) when menstrual period became regular (<12, 13–14, 15–16, or 17+), number of live births (none, 1–2, 3–4, or 5+), family history of breast cancer (yes or no), and daily alcohol drinking (yes or no). Additional adjustment for BMI (weight in kg divided by height in meters squared) did not materially alter any study results; therefore, all of the figures presented in the report were derived from models without adjustment for BMI.

Statistical analysis was carried out using the SAS software version 8.2 (SAS Institute, Cary, NC) and Epilog for Windows Version 1.0 (Epicipher Software, Pasadena, CA). All of the Ps reported are two-sided, and Ps of
RESULTS

Breast cancer cases and controls were similar in distribution by dialect group (48.1% versus 50.2% Cantonese in case and control groups, respectively). Cases were more educated than controls; 29% of cases had no formal education compared with 39% of controls. Hence, all of the subsequent case-control analyses were adjusted for level of education.

Table 1 shows the distribution of known risk factors for breast cancer in the study population. Late age at menarche (≥17 years) and increasing number of live births were associated with a significant reduction in breast cancer risk. As expected, nulliparity, late age at first live birth, family history of breast cancer, daily alcohol drinking, late age at menopause, and a high BMI (28 + kg/m²) in postmenopausal subjects were positively associated with risk of breast cancer. Very few subjects used replacement hormones, and there was no association between such use and breast cancer risk.

Table 2 shows the distribution of ACE genotypes among study subjects. There was a statistically significant linkage disequilibrium between the I/D and A-240T polymorphisms (contingency coefficient = 0.74; P < 0.001). Among the controls, frequencies for the DD, ID, and II genotypes were 0.09, 0.47, and 0.44, respectively. For the A-240T polymorphism, frequencies for the TT, AT, and AA genotypes were 0.10, 0.49, and 0.41, respectively.

Table 3 shows that the risk of breast cancer was highly influenced by the two ACE polymorphisms. For the A-240T polymorphism, frequency of the TT genotype was 15.8% among cases but only 9.7% among controls. Women with one copy of the low activity A allele showed a reduced risk of breast cancer compared with those homozygous for the T allele. Having two copies of the A allele was not associated with an additional reduction in breast cancer risk. After adjustment for breast cancer risk factors (age at menarche, number of live births, family history of breast cancer, and daily alcohol drinking), we observed a statistically significant 45% reduction in risk of breast cancer among women with the AT/TT genotype relative to those with the TT genotype (OR, 0.55; 95% CI, 0.34–0.90). A similar risk reduction was observed when the analysis was confined to invasive cancers only (OR, 0.53; 95% CI, 0.34–0.90). Comparable results were obtained with respect to the ACE I/D polymorphism (Table 3). When the I/D and A-240T genotypes were considered simultaneously, women with both A and I alleles (the low-activity genotype) had a statistically significant reduction of close to 50% in breast cancer risk compared with those carrying either the TT and/or DD genotypes (the high-activity genotype; Table 3).

ACE inhibitors are commonly prescribed to patients with hypertension, heart disease, or diabetes, and usage of such drugs among our study subjects could potentially confound the ACE genotype-cancer associations. To minimize this possible confounding effect, we repeated the analysis after excluding all of the subjects with history of hypertension, heart disease, or diabetes (use of ACE inhibitors was not specifically solicited during the baseline interview; Table 4). Frequency of the TT genotype was 16.9% among cases and 9.1% among controls, and that of the DD genotype was 15.3% among cases and 8.4% among controls. Compared with results based on all of the study subjects, all of the genotype-cancer associations became stronger (Table 4).

We repeated the analysis on all of the study subjects stratified by stage of disease. There was no evidence that the ACE genotype-breast cancer association differed between localized versus advanced disease (data not shown). Results also remained similar when the 17 cases of in situ cancers or unknown stage were excluded (data not shown).

DISCUSSION

To our knowledge, this is the first study that examines the relationship between the ACE gene polymorphism and breast cancer risk. We demonstrate that individuals carrying genotypes that predispose them to lower plasma concentrations of this enzyme have a significantly reduced risk of breast cancer, and this asso-

<ref>Table 1</ref> Distribution of known risk factors for breast cancer in Singapore Chinese women, the Singapore Chinese Health Study

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Cases</th>
<th>Controls</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family history of breast cancer</td>
<td>No</td>
<td>Yes</td>
<td>1.86 (1.16–2.97)</td>
</tr>
<tr>
<td>Daily alcohol drinker</td>
<td>No</td>
<td>Yes</td>
<td>2.63 (1.23–5.65)</td>
</tr>
<tr>
<td>Age (yrs) at menopause (women ≥55 years only)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>≥40</td>
<td>31</td>
<td>1.00 (0.73–1.38)</td>
</tr>
<tr>
<td>BMI (kg/m²) (postmenopausal women only)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>≥20</td>
<td>13</td>
<td>1.00 (0.68–1.48)</td>
</tr>
<tr>
<td>Use of replacement hormone</td>
<td>No</td>
<td>180</td>
<td>1.00 (0.93–1.08)</td>
</tr>
<tr>
<td>Ex</td>
<td>2</td>
<td>8</td>
<td>1.03 (0.21–5.00)</td>
</tr>
<tr>
<td>Current</td>
<td>7</td>
<td>31</td>
<td>0.80 (0.34–1.86)</td>
</tr>
</tbody>
</table>

<ref>Table 2</ref> Distribution of ACE genotypes among all of the study subjects,<sup>a</sup> the Singapore Chinese Health Study

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases</th>
<th>Controls</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>67</td>
<td>21</td>
<td>1.00 (0.73–1.38)</td>
</tr>
<tr>
<td>AT</td>
<td>11</td>
<td>329</td>
<td>1.00 (0.88–1.15)</td>
</tr>
<tr>
<td>AA</td>
<td>0</td>
<td>26</td>
<td>0.53 (0.34–0.80)</td>
</tr>
</tbody>
</table>

<sup>a</sup> D and T are the high-activity alleles; 52 subjects with noninformative I/D or A-240T genotype were excluded from this analysis.
We noted a high degree of linkage between the I/D and A240-T polymorphisms, consistent with prior data (2). The frequency of the putative low-risk I allele is 0.68 and that of the high-risk DD genotype is 0.09 in our control population. This is consistent with the frequencies reported previously for Singapore Chinese (32), which are similar in Japanese (33) and other Chinese populations in Asia (34, 35).

In the present study, we had considered the possibility that subjects with the DD genotype were more likely to have used ACE inhibitors compared with those possessing the other genotypes. This may arise because DD homozygosity has been associated with increased risk of hypertension (3–5), atherosclerotic cardiovascular complications, and diabetic nephropathy (36), conditions for which ACE inhibitors are commonly prescribed. These medications lower the subject’s ACE levels and, thus, may attenuate the risk estimates in the study. When we excluded subjects with these medical conditions from the analysis, the risk reductions associated with the low-activity alleles were enhanced, thus reinforcing the inference that individuals with genetically lower levels of ACE have reduced susceptibility to breast cancer.

Our strategy of examining functional ACE polymorphisms to test the hypothesis that elevated ACE levels predispose to the development of breast cancer has several advantages over previous observational studies, which examined use of ACE inhibitors alone (20–23). One likely reason for their contradictory results is the lack of adjustment for ACE genotype in these prior studies. In addition, disagreement between these studies may stem from variation in choices of ACE inhibitors across the study populations, as different ACE inhibitors have shown various in vitro efficacy in inhibiting endothelial cell migration in the process of angiogenesis (19). It is also difficult to assess and control for factors such as compliance, dosage, and duration of the drug prescription, all of which can lead to misclassification and attenuation of risk estimates in the studies.

The current study has several strengths. Singapore is a small city-state where there is good access to specialized medical care. The nationwide cancer registry has been in place since 1968 and has been shown to be comprehensive in its recording of cancer cases (37). Thus, breast cancer case ascertainment can be assumed to be complete. Our study subjects originated from two contiguous regions in South China, leading to a high degree of genetic homogeneity. The study allows for the adjustment of known environmental risk factors for breast cancer, all of which were assessed before cancer diagnosis and, thus, can be presumed to be free of recall bias.

The chief limitation of our study is the lack of information on use of specific ACE inhibitors, which may lead to underestimate of the protective effect of the polymorphisms of the ACE gene on breast cancer risk (see earlier discussion of DD genotype being linked to increased risks of hypertension, cardiovascular disease, and diabetic nephropathy). In fact, in the present study, exclusion of subjects with conditions that commonly require prescriptions for ACE inhibitors led to stronger associations between the ACE genetic polymorphisms and breast cancer risk. Another weakness of the present study was our relatively few cancer cases, which

| Table 3 ACE genotypes in relation to risk of breast cancer, the Singapore Chinese Health Study |
|---------------------------------------------|------------------|------------------|------------------|
| A-240T genotype^c                        | Cases          | Controls       | OR^a (95% CI)   | OR^b (95% CI)   |
| TT                                        | 29             | 63             | 1.00            | 1.00            |
| AT                                        | 79             | 318            | 0.54 (0.32–0.90)| 0.51 (0.30–0.85)|
| AA                                        | 76             | 271            | 0.64 (0.38–1.07)| 0.60 (0.36–1.02)|
| AT or AA                                  | 155            | 589            | 0.59 (0.36–0.95)| 0.55 (0.34–0.90)|
| ID genotype^c                            |                |                |                 |                 |
| DD                                        | 23             | 56             | 1.00            | 1.00            |
| ID                                        | 79             | 282            | 0.70 (0.40–1.21)| 0.66 (0.38–1.16)|
| ID or II                                  | 159            | 587            | 0.67 (0.40–1.13)| 0.63 (0.37–1.07)|

a Adjusted for age at recruitment, year of recruitment, dialect group, and level of education.
b Additionally adjusted for age when period became regular, number of live births, family history of breast cancer, and daily alcohol drinking.
c Sum did not add up to total subjects because of missing values (see “Materials and Methods” for details).

| Table 4 ACE genotypes in relation to risk of breast cancer after exclusion of subjects with history of hypertension, heart diseases, or diabetes,^a the Singapore Chinese Health Study |
|---------------------------------------------|------------------|------------------|------------------|
| A-240T genotype^c                        | Cases          | Controls       | OR^a (95% CI)   | OR^b (95% CI)   |
| TT                                        | 21             | 43             | 1.00            | 1.00            |
| AT                                        | 49             | 230            | 0.46 (0.25–0.85)| 0.42 (0.23–0.80)|
| AA                                        | 54             | 201            | 0.60 (0.32–1.10)| 0.58 (0.31–1.06)|
| AT or AA                                  | 103            | 431            | 0.52 (0.29–0.93)| 0.49 (0.27–0.89)|
| ID genotype^c                            |                |                |                 |                 |
| DD                                        | 19             | 39             | 1.00            | 1.00            |
| ID                                        | 46             | 220            | 0.47 (0.24–0.89)| 0.45 (0.23–0.86)|
| ID or II                                  | 95             | 407            | 0.48 (0.28–0.83)| 0.46 (0.27–0.81)|

^a Sixty cases and 183 control subjects were excluded from this analysis.
b Adjusted for age at recruitment, year of recruitment, dialect group, and level of education.
c Additionally adjusted for age when period became regular, number of live births, family history of breast cancer, and daily alcohol drinking.
d Sum did not add up to total subjects because of missing values (see “Materials and Methods” for details).
resulted in less precise estimation of RRs for breast cancer associated with genetic and environmental risk factors.

Our findings of an inverse association between low-activity ACE alleles and breast cancer risk support the involvement of the renin-angiotensin system in breast carcinogenesis, and are consistent with recent experimental data. The two different receptors that bind angiotensin II, designated as angiotensin II type I receptor and angiotensin II type II receptor (38, 39), have been identified in the epithelial cells of the ducts and lobules in normal tissues, as well as in benign and malignant tumors of the human breast (40, 41). Angiotensin II has been demonstrated to stimulate proliferation in a human breast adenocarcinoma cell line via the angiotensin II type I receptor, and this growth effect may be involved in the pathogenesis of premalignant lesions (42). Through its action via the angiotensin II type II receptor, angiotensin II may induce increased production of nitric oxide (40), which in turn has shown to augment tumor growth and metastasis in a murine breast cancer model (43). Angiotensin II may also facilitate metastasis by inducing cell adhesion factors in breast cancer (44).

In summary, the present study lends support to limited prior evidence that ACE activity/level is etiologically linked to breast cancer development. Our observations have practical implications in prevention and treatment strategies for breast cancer. If lower ACE level/activity is indeed protective against breast cancer, then use of ACE inhibitors instead of drugs via other pathways to control hypertension and related disease should be encouraged. More importantly, the development of chemopreventive agents targeting this putative pathway, and subsequent administration of such drugs to individuals at high risk for breast cancer, may lessen the population burden of breast cancer morbidity and mortality.

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