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Genetic Variation in Angiotensin I-Converting Enzyme (*ACE*) and Breast Cancer Risk: The Multiethnic Cohort[1]

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ABSTRACT

The *A-240T* and *I/D* polymorphisms in the angiotensin I-converting enzyme (*ACE*) gene are markers of circulating ACE levels and have been associated with numerous cardiovascular disease outcomes. More recently, the low-activity *A* and *I* alleles at these polymorphic sites have been inversely related with breast cancer risk. We assessed the relationship between the *A-240T* and *I/D ACE* variants and breast cancer risk in a case-control analysis ($n = 1263$ cases with invasive breast cancer and 2269 controls) among African-American, Japanese, Latina, and white women in the Multiethnic Cohort Study. Odds ratios and 95% confidence intervals are presented adjusted for established breast cancer risk factors. Among all women combined, we observed no significant association between the *A-240T* polymorphism and breast cancer risk. For the *I/D* polymorphism, contrary to expectation, women with the *I/I* genotype had a marginally significant increase in breast cancer risk (*versus DD* genotype: odds ratio, 1.30; 95% confidence interval, 1.05–1.61), although associations were not entirely consistent across ethnic groups. These data do not support the hypothesis that women with lower ACE levels, as predicted by the low-activity *A* and *I ACE* alleles, are at reduced risk of breast cancer. Overall, these results suggest that the *A-240T* and *I/D ACE* polymorphisms are not likely to be strong predictors of breast cancer risk.

INTRODUCTION

Angiotension II is a key regulator of blood pressure homeostasis in humans and is produced from angiotensin I by ACE.[3] Plasma ACE concentrations vary between individuals, and a substantial proportion of the interindividual difference in ACE levels is governed by genetic variation at the *ACE* locus (1). Two polymorphisms in the *ACE* gene, a 287-bp *Alu* insertion/deletion (*I/D*) polymorphism in intron 16 and the *A-240T* polymorphism in the 5′-flanking region, have been associated with circulating ACE levels (1, 2). Studies subsequently evaluating the *I/D* polymorphism as a marker of blood pressure and risk of myocardial infarction, however, have provided inconsistent results (3–7).

An etiologic relationship between hypertension and hormone-related cancer incidence has been suggested. Hypertension during pregnancy and after menopause has been positively associated with breast cancer risk in a small number of studies (8, 9). Women using ACE inhibitors and other antihypertensive medications have been reported to have lower risks of breast cancer, although study findings have not been entirely consistent (10–12). There are new data to support the direct involvement of angiotensin II in breast cell proliferation (13), angiogenesis, and tumor metastasis (14), key processes implicated in breast cancer development and progression. Based on the prior evidence linking ACE activity and angiotensin II with breast cancer, Koh *et al.* (15) hypothesized that women carrying the low-activity (*A* and *I*) alleles of the *ACE A-240T* and *I/D* polymorphisms would have lower ACE levels and decreased synthesis of angiotensin II and, consequently, would be less susceptible to developing breast cancer. In a breast cancer case-control study among Chinese women from Singapore ($n = 189$ cases), the authors reported that women with the low-activity genotypes were at decreased risk of breast cancer (*ID + II* genotypes *versus DD*: OR, 0.63; 95% CI, 0.37–1.07; *AT + AA* genotypes *versus TT*: OR, 0.55; 95% CI, 0.34–0.90; Ref. 15).

In the present study we have evaluated the *A-240T* and *I/D* polymorphisms in the *ACE* gene in relation to breast cancer risk among African-American, Japanese, Latina, and white women in a large nested case-control in the MEC. We also characterized the degree of LD between the two polymorphisms in each ethnic group.

MATERIALS AND METHODS

The MEC consists of 215,251 men and women in Hawaii and Los Angeles and has been described in detail elsewhere (16). In brief, the cohort is comprised predominantly of Hawaiian, Japanese, and white residents living in Hawaii and African-Americans and Latinos living in Los Angeles. Between 1993 and 1996, participants entered the MEC by completing a 26-page self-administered mail questionnaire that asked detailed information about dietary habits, demographic factors, personal behaviors, history of prior medical conditions (*e.g.*, heart attack, diabetes, and cancer), current medication use, family history of common cancers, and for women, reproductive history and exogenous hormone use (HRT). Potential cohort members were identified primarily through the Department of Motor Vehicles drivers' license files in Hawaii and California and, additionally for African-Americans, Health Care Financing Administration data files. The participants were between the ages of 45 and 75 years when they entered the cohort.

Eligible cases in this nested breast cancer case-control study consisted of African-American, Japanese, Latina, and white women with incident breast cancer (including second primaries) diagnosed after enrollment in the MEC through May 2002. Incident cancers in the MEC are identified by cohort linkage to population-based Surveillance, Epidemiology and End Results (SEER) cancer registries in Hawaii and Los Angeles and to the California State cancer registry. Information on stage of disease at the time of diagnosis is also collected from the cancer registries. Women with nonlocalized tumors were classified as having advanced disease. Beginning in 1995, blood sample collection was initiated from incident breast cancer cases, including those diagnosed since recruitment into the cohort. At this time, blood collection was also initiated from a random sample of the cohort within each ethnic group to serve as controls for genetic analyses in the cohort. The participation rates for providing a blood sample are 74% and 66% for cases and controls, respectively. Controls in this study were women without breast cancer before entry into the cohort and without a diagnosis up to May 2002. The breast cancer case-control study consists of 1263 breast cancer cases (316 with advanced disease) and 2269 controls. This study was approved by the Institutional Review Boards at the University of Southern California and at the University of Hawaii.

Laboratory Methods. DNA was extracted from WBC fractions using the Qiagen DNA Blood Kit (Qiagen, Valencia, CA). Genotyping was performed by the 5′ nuclease TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA) using the protocol described by Koh *et al.* (15). Laboratory personnel were blinded to case-control status, and quality control samples (5%)

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[3] The abbreviations used are: ACE, angiotensin I-converting enzyme; MEC, Multiethnic Cohort Study; OR, odds ratio; CI, confidence interval; HRT, hormone replacement therapy; LD, linkage disequilibrium.

were inserted to validate genotyping procedures. The concordance rates for the blinded samples were 99% and 97% for the *A-240T* and *I/D* polymorphisms, respectively. Subjects missing genotype information were removed from variant specific analyses [*A-240T*, $n = 294$ (8.3%); *I/D*, $n = 408$ (11.6%)].

Statistical Analysis. Because of the initially delayed but thereafter ongoing collection of samples from cases and controls, we have adopted a standard case-control approach to statistical analysis. In this analysis, we adjusted for age by equating the age at diagnosis of the case to the age at blood draw of the control. No adjustment was made for calendar year of blood draw because the cases were collected over a relatively short time period of 7 years. Eighty-eight controls became cases after their blood draw as a control; these women were included in the analysis as cases with age at diagnosis as the age variable (other approaches to deal with these cases did not alter the results because they comprise such a small fraction of the total cases).

LD between the *A-240T* and *I/D* alleles was evaluated among controls for each ethnic group using the D′ statistic (17). ORs and 95% CIs were calculated for cases and controls using unconditional logistic regression. In addition to age (<55 years, 55–64 years, ≥65 years) we also adjusted for the established breast cancer risk factors: age at menarche (≤12 years, 13–14 years, ≥15 years), parity (nulliparous, 1 child, 2–3 children, 4+ children), menopausal status/HRT use [five categories: (*a*) premenopausal women; (*b*) postmenopausal women (women with a natural menopause or bilateral oophorectomy)/never user of HRT; (*c*) postmenopausal women/past HRT user; (*d*) postmenopausal women/current HRT user; and (*e*) women with an unknown menopausal or unknown HRT user status], history of breast cancer in a first-degree family relative (yes/no), and current alcohol consumption (0 drinks/day, 1–2 drinks/day, 3+ drinks/day). Body mass index was not a confounder in the analysis and was not included in the multivariate models. Controlling for self-reported history of high blood pressure also did not alter the results. For comparison with the data of Koh *et al.* (15), polymorphisms were evaluated using the *TT* (*A-240T*) and *DD (I/D*) hypothesized high-risk genotypes as the reference categories. Analyses were stratified by ethnicity, and a summary OR was estimated controlling for age*ethnicity and the established breast cancer risk factors. We also evaluated associations among women not likely to be users of ACE inhibitors. We defined users as women reporting a previous history of high blood pressure or any use of high blood pressure medication ($n = 1549$). All controls were included in analyses by stage of disease (localized or advanced). One case missing age at diagnosis was removed from all analyses. We used the SAS statistical package version 8.2 for all analyses (SAS Institute, Cary, NC).

RESULTS

Among all women, the mean age of the cases and controls was 64.3 and 63.4 years, respectively. The mean age was similar for cases and controls within each ethnic group (Table 1). The distributions of established breast cancer risk factors were generally consistent with expectation and were consistent with what we observed in the overall cohort (18). Compared with controls, cases were more likely to be a current or past user of HRT and to have a first-degree family history of breast cancer. Cases were also more likely to be nulliparous and to have had children at a later age. These associations were generally consistent across all ethnic groups (Table 1).

Among controls, the frequency of the *A* allele of the *A-240T* polymorphism was comparable between ethnic groups (range, 0.61–0.68). There was considerable ethnic variation in the frequency of the *I* allele of the *ACE I/D* polymorphism; the prevalence was 40%, 46%, 52%, and 66% among African Americans, whites, Latinas, and Japanese, respectively. Genotype frequencies were in Hardy-Weinberg equilibrium among controls in each ethnic group (data not shown). Evidence for LD between the *A* and *I* alleles was strongest for the

Table 1 *Descriptive characteristics of breast cancer cases (n = 1263) and controls (n = 2269) in the MEC*

	Ethnicity							
	African Americans		Japanese		Latinas		Whites	
	Cases ($n = 278$)	Controls ($n = 672$)	Cases ($n = 358$)	Controls ($n = 429$)	Cases ($n = 272$)	Controls ($n = 706$)	Cases ($n = 355$)	Controls ($n = 462$)
Age (mean) (yrs)	64.1	64.3	64.4	64.2	63.9	62.8	64.7	62.3
Menopausal status (%)								
Premenopausal	14	11	13	21	10	11	8	20
Postmenopausal[a]	55	56	67	62	66	61	66	61
Simple hysterectomy	19	23	10	10	14	19	17	14
Missing	11	10	11	8	10	9	8	5
HRT use (%)[a,b]								
Never	47	48	28	29	44	48	28	34
Past	29	23	16	15	21	19	17	17
Current	21	26	56	53	31	28	55	48
Age at menarche (%)[b] (yrs)								
≤12	54	46	56	50	48	49	55	48
13–14	34	39	32	35	37	39	35	44
15+	12	14	9	14	13	11	8	8
No. of children (%)[b]								
0	12	11	16	11	10	7	18	16
1	20	16	11	10	8	6	11	9
2 or 3	40	40	54	60	36	36	51	53
4+	25	32	17	18	45	50	19	22
Age at first birth (%)[b,c] (yrs)								
<20	46	50	8	11	35	42	23	23
21–30	44	41	74	75	53	52	66	63
31+	8	5	14	12	9	4	10	12
First-degree family history of breast cancer (%)[b]								
Yes	23	12	18	11	17	10	15	9
No	72	82	77	87	75	83	81	88
Average alcohol consumption (drinks/day)[b]								
0	51	53	72	75	54	53	31	39
<1	29	30	19	17	32	33	39	40
≥1	10	10	5	3	5	6	22	18

[a] Women reporting natural menopause or having had a bilateral oophorectomy.
[b] Percentages do not add up to 100% because of missing data.
[c] Among parous women.

Japanese (D′ = 0.95), Latinas (D′ = 0.91), and whites (D′ = 0.89) and less so for the African Americans (D′ = 0.19).

Among all women combined, we observed no significant association between *A-240T* genotype and risk of breast cancer (Table 2); compared with the *TT* genotype, the adjusted OR for *A/A* homozygotes was 1.05 (95% CI, 0.83–1.33). Associations were not consistent across ethnic groups. Compared with women with the *TT* genotype, African-American women homozygous for the *A* allele were at lower risk (OR, 0.59; 95% CI, 0.37–0.94; *P* for trend, 0.02), whereas a significant positive association was observed for Latinas (OR, 1.91; 95% CI, 1.07–3.41; Table 2). We observed a modest positive association between the *I* allele and breast cancer risk (*ID versus DD* genotype: OR, 1.16; 95% CI, 0.96–1.41; *II versus DD* genotype: OR, 1.30; 95% CI, 1.05–1.61; *P* for trend, 0.02; Table 2). Evidence of this association was strongest among the African Americans (*II versus DD* genotype: OR, 1.76; 95% CI, 1.15–2.68) and noted in all groups except for the Japanese (*II versus DD* genotype: OR, 1.04; 95% CI, 0.62–1.75). Results were similar when restricting the analysis to women with advanced disease and when limiting the analysis to women classified as not using ACE inhibitors (data not shown).

DISCUSSION

Among all women in this study, carriers of the *A* or *I* alleles of the *A-240T* and *I/D ACE* polymorphisms were not at decreased risk of breast cancer. However, we did observe a modest positive association between the *I/I ACE* genotype and breast cancer risk, although this association was not observed consistently across all ethnic groups. Only one other study has evaluated the relationship between variation at the *ACE* locus and breast cancer risk. Among 189 cases and 671 controls within a Chinese cohort, Koh *et al.* (15) observed that women with either the *A* or *I ACE* allele were at significantly decreased risk of breast cancer (*A* allele: OR, 0.55; 95% CI, 0.34–0.90; *I* allele: OR, 0.63; 95% CI, 0.37–1.07). Associations were strongest for women carrying either "low-risk" allele (OR, 0.52; 95% CI, 0.33–0.84) and among the subgroup of women ($n = 129$ cases) classified as not likely to be using ACE inhibitors (OR, 0.46; 95% CI, 0.27–0.81). Overall, our findings do not support those presented by Koh *et al.* (15) suggesting that the *A* and *I* alleles may serve as protective markers of breast cancer risk.

In our study, the prevalence of these two polymorphisms in the different ethnic groups was similar to those reported in other studies (2, 15, 19). Among the Japanese controls, the frequencies of the *A* allele (63%) and *I* allele (66%) were nearly identical to those observed among the Chinese women in the study by Koh *et al.* (Ref. 15; *A* allele, 66%; *I* allele, 68%). We observed considerable variability in LD between these two markers across ethnic groups; the *A* and *I* alleles were more tightly linked among the Japanese, Latinas, and whites and in almost complete linkage equilibrium among the African Americans. These data are consistent with known differences in the extent of LD across the *ACE* locus between African and non-African populations as reported by other studies (20, 21).

Segregation and linkage studies have provided strong support for an *ACE*-linked quantitative trait locus (2, 22, 23), although the underlying functional variants involved in predicting differences in ACE levels have not been defined. In an initial study in a white European population, individuals with the *A* allele of the *A-240T* polymorphism were observed to have lower ACE levels (2). Subsequent studies have not confirmed this finding (24, 25). A more recent study among an African population reported the *A* allele to be associated with increased ACE concentration (25). Associations have been more consistently observed with the *I/D Alu* polymorphism in intron 16, with the *I* allele demonstrated to be a marker of lower circulating ACE (1, 2, 22). In a white European population, the majority of variation in ACE levels has been localized to an ~18-kb region located between two ancestral recombination sites in the *ACE* gene (26). The high degree of LD observed between the *I/D* polymorphism and dozens of other variants in this region has hampered efforts to undercover the functional variant underlying interindividual variation in circulating ACE levels in white populations. Cladistic haplotype analyses of the ACE gene have provided evidence that the *I/D* variant is not likely to be the underlying functional polymorphism (19, 27). In a study among Nigerians, Cox *et al.* (19) observed significant differences in ACE levels between individuals with haplotypes harboring the *I* allele, suggesting that this allele may be a marker of low ACE levels in some populations, but not all populations. Differences in LD between populations may also explain the inconsistent associations that we observed with the *A-240T* polymorphism between African Americans and the other groups.

Genetic associations initially reported in small studies are rarely replicated in subsequent studies (28). A strength of the present study is the large sample size (>270 cases) among each of four ethnic populations. This study design enables the reproducibility of an association to be evaluated across multiple ethnic groups, providing more convincing support for an underlying relationship between a genetic marker and breast cancer risk. Our sample size within each ethnic group, however, is not large enough to definitively evaluate ethnic-specific risks. These data provide little support for the hypothesis that the *A A-240T ACE* allele is an independent risk factor for

Table 2 *Associations between ACE A-240T and I/D polymorphisms and breast cancer risk in the MEC*

	African Americans			Japanese			Latinas			Whites			All Groups Combined
	Cases	Controls	OR (95% CI)[a]	Cases	Controls	OR (95% CI)[a]	Cases	Controls	OR (95% CI)[a]	Cases	Controls	OR (95% CI)[a]	OR (95% CI)[b]
A-240T genotype[c]													
TT	42	78	1.00	43	56	1.00	17	78	1.00	48	70	1.00	1.00
AT	116	276	0.75 (0.48–1.19)	159	180	1.21 (0.76–1.94)	109	267	1.88 (1.06–3.34)	128	195	0.96 (0.61–1.52)	1.10 (0.87–1.39)
AA	90	280	0.59 (0.37–0.94)	125	155	1.12 (0.70–1.82)	124	312	1.91 (1.07–3.41)	129	161	1.14 (0.72–1.80)	1.05 (0.83–1.33)
P trend			0.02			0.82			0.12			0.44	0.88
AT + AA	206	556	0.67 (0.44–1.03)	284	335	1.17 (0.75–1.83)	233	579	1.89 (1.09–3.30)	257	356	1.05 (0.69–1.60)	1.08 (0.86–1.34)
I/D genotype[c]													
DD	77	221	1.00	37	43	1.00	49	162	1.00	84	124	1.00	1.00
ID	118	310	1.12 (0.79–1.58)	128	160	1.06 (0.63–1.79)	127	301	1.38 (0.93–2.04)	129	187	1.01 (0.70–1.47)	1.16 (0.96–1.41)
II	62	100	1.76 (1.15–2.68)	119	154	1.04 (0.62–1.75)	73	189	1.29 (0.84–1.97)	79	91	1.42 (0.92–2.18)	1.30 (1.05–1.61)
P trend			0.01			0.96			0.30			0.13	0.02
ID + II	180	410	1.28 (0.93–1.77)	247	314	1.05 (0.64–1.72)	200	490	1.34 (0.93–1.94)	208	278	1.14 (0.81–1.61)	1.21 (1.01–1.45)

[a] Unconditional logistic regression adjusted for age, age at menarche, parity, menopausal status/HRT use, first-degree family history of breast cancer, and current alcohol consumption.

[b] Unconditional logistic regression adjusted for age, ethnicity, age at menarche, parity, menopausal status/HRT use, first-degree family history of breast cancer, and current alcohol consumption.

[c] Numbers do not add to the total number of subjects because of missing genotype data.

breast cancer. We observed a suggestive positive association between the *I/I ACE* genotype and breast cancer risk; however, the association was not observed among all ethnic groups. Additional studies exploring the LD block structure and haplotype patterns among these four populations are needed to clarify the contribution of genetic variation in *ACE* to disease risk.

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