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Advances in Brief

Polymorphism in the Androgen Receptor and Mammographic Density in Women Taking and Not Taking Estrogen and Progestin Therapy[1]

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Abstract

There is some evidence that women with a higher number of CAG repeat lengths on the androgen receptor (*AR*) gene have increased breast cancer risk. We evaluated the association between *AR*-CAG repeat length and mammographic density, a strong breast cancer risk factor, in 404 African-American and Caucasian breast cancer patients. In postmenopausal estrogen progestin therapy users, carriers of the less active *AR*-CAG had statistically significantly higher mean percentage of density (41.4%) than carriers of the more active *AR*-CAG (25.7%; $P = 0.04$). Our results raise the question of whether the number of *AR*-CAG repeats predicts breast cancer risk in estrogen progestin therapy users.

Introduction

Evidence suggests that circulating androgen levels in postmenopausal women are associated with breast cancer risk (1). In a pooled analysis of nine prospective studies of postmenopausal women, the trend in breast cancer risk associated with increasing level of testosterone was statistically significant. The main receptor for testosterone and dihydrotestosterone in breast tissue is the androgen receptor (AR). Within the first exon of the *AR* gene is a polymorphic CAG repeat. Several epidemiological studies have examined the association between the length of the CAG repeat polymorphism in the *AR* gene and breast cancer risk (2–8). Three studies have observed that women with the less active *AR* allele (long CAG repeat) have increased breast cancer risk (3, 5, 7), although the evidence is not entirely consistent (2, 4, 6, 8). Only one study has examined the association between the *AR* genotype and mammographic density, and no association was observed (9). This apparent contradiction, that high androgen levels but the less active *AR* genotype are associated with breast cancer risk is not well understood. To shed more light on the *AR*-breast cancer association, we examined the association between the *AR*-CAG repeat length and mammographic density, a strong and independent breast cancer risk factor (10–12). We previously reported results demonstrating that mammographic density represents a strong risk factor for breast cancer in this study (12), and furthermore, that genes important in estrogen metabolism do not explain the variation in mammographic density observed in these women (13).

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Materials and Methods

Study Population. Study subjects were women newly diagnosed with a first primary invasive breast cancer who participated in the Los Angeles part of the Women's Contraceptive and Reproductive Experiences (CARE) Study ($n = 1242$; Ref. 14). The Women's CARE Study was a population-based case-control study of invasive breast cancer in United States-born African-American and Caucasian women aged 35–64 years. All of the patient participants were diagnosed between July 1994 and April 1998. Each participant provided a written informed consent at the time of interview. All of the women were interviewed in person by trained interviewers using a standardized questionnaire to collect information on demographic factors and potential breast cancer risk factors including histories of oral contraceptive use, hormone therapy (HT) use, family history of breast cancer, age at menarche, complete pregnancy history, lifetime history of participation in physical exercise activities, and mammogram history during the five years preceding the patient's date of diagnosis. We restricted eligibility for this study to breast cancer patients who reported having a screening mammogram in the five years before breast cancer diagnosis. We requested screening mammograms for 60.9% of the patients included in the Los Angeles portion of the CARE study and who provided written permission for the release of mammograms ($n = 755$, 426 Caucasian-Americans and 329 African-Americans). We received mammograms for 71.1% ($n = 303$) of the eligible Caucasian patients and 69.3% ($n = 228$) of eligible African-American patients (12). During the conduct of the Women's CARE Study in Los Angeles County, we collected a blood sample from all of the patients willing to provide a sample. Of the 531 cases for whom we had a screening mammogram, we had a blood sample for 423 (79.7%) women (13). The study protocol was approved by the Institutional Review Board at the University of Southern California.

Genotyping. Using a standard protocol, genomic DNA was extracted from peripheral blood (13). DNA samples were sent to City of Hope Beckman Research Institute for *AR* genotyping. The exon 1 CAG repeat of the *AR* was amplified by PCR using a fluorescently labeled forward primer (5′-TCCAGAATCTGTTCCAGAGCGTGC-3′) and reverse primer (5′-GCTGTGAAGGTTGCTGTTCCTCAT-3′). All of the microsatellite genotyping was performed using an ABI 377 sequencer with Genescan and Genotyper software (PE/Applied Biosystems, Foster City, CA). The number of CAG repeats was calculated based on direct sequencing results of 8 prostate cancer cases with different known PCR product lengths.

Mammographic Density. We measured percentage of mammographic density using the University of Southern California Madena computer-based threshold method of assessing density (15). Percentage of mammographic density in the right and left breasts of both case and control women have been shown to be highly correlated (16). Therefore, we used screening mammograms of the contralateral (nondiseased) breast obtained at or before diagnosis, The cranio-caudal mammographic images were digitized using a high-resolution Cobrascan CX-312T scanner (Radiographic Digital Imaging, Torrance, CA) and then viewed on a computer screen. A single reader blinded to all of the patient characteristics evaluated all of the images. The reader first defined the total breast area using a special outlining tool. The density assessments were done as follows. The reader defined the region of interest (ROI) excluding the pectoralis muscle and other light artifacts (prominent veins, and so forth). The reader then applied a yellow tint to gray levels above a selected threshold using a computerized tool. The area highlighted in this manner was considered to represent dense tissue. The software then calculated the number

of pixels within the entire breast as outlined and the number of pixels tinted yellow within the ROI. The ratio of the number of tinted pixels within the ROI to the total number of pixels in the breast represents the measure of percentage of density.

The scanned mammogram files of 15 women could not be assessed for density, because the digitized files were unusable and we were unable to obtain the films a second time. We excluded the mammograms of 4 women who had only one mammogram and were pregnant at the time of mammogram. Therefore, we obtained mammographic density results for 404 breast cancer patients for whom we had a blood sample.

Menopausal Status/HT Use Status at Mammography. Women were assigned a menopausal status at the time of their mammogram. Women who had menstruated and not used HT within 3 months before mammography were defined as premenopausal. Women who had not menstruated within 3 months before a mammogram, who had a bilateral oophorectomy >3 months before a mammogram, who had a simple hysterectomy before a mammogram with the last menstrual period >6 months before surgery, who were 50 years of age or older and were current or past HT users, or who were 60 years of age or older were defined as postmenopausal. Otherwise, menopausal status was considered as unknown.

Postmenopausal women were additionally categorized based on their HT usage as never, past, or recent (within the past 5 years). Type of HT used was additionally specified as estrogen therapy (ET) or estrogen progestin therapy (EPT). Women who had used both ET and EPT were assigned their most recent HT regimen.

Statistical Methods. We classified each allele of the *AR* gene as short (S) or long (L) using the median number of CAG repeats across all of the alleles in the study population, 21 repeats, as the cut-point. Each woman was categorized by genotype as S/S, S/L, or L/L. We used least squares linear regression methods to model the dependence of percentage of mammographic density on *AR* genotypes. All of the models included adjustment for the following set of potential confounders selected *a priori* based on their previously reported association with density and/or the *AR* genotype, age at mammogram (years): 35–39, 40–44, 45–49, 50–54, 55–59, or 60–64; body mass index (kg/m^2): <22, 22–24.9, 25–29.9, or ≥30; and race: African-American or Caucasian.

In addition to using linear regression, we also modeled percent density as a categorical outcome using ordinal logistic regression methods. A four-category variable was created representing quartiles of percent density based on the distribution of percent density (range, 00.0–85.7%) in the study population. We calculated odds ratios (ORs) to estimate the odds of having a single quartile higher level in percentage of mammographic density associated with the *AR* genotype. In addition to analyzing the entire sample, both linear and logistic regression models were applied to subgroups defined by race, family history of breast cancer (mother or sister), menopausal status, age at mammogram, and HT status to examine possible effect modification. Data were analyzed using SAS v9 software (SAS Institute Inc., Cary, NC).

Table 1 *Mean percentage of mammographic density by descriptive characteristics (n = 404)*

Characteristic	*n*	%	Mean % density	*P*[a]
Race[b]				
African-American	158	39.1	34.1	
Caucasians	246	60.9	34.3	0.95
Menopausal status/HT[c] use				
Premenopausal	191	47.2	35.6	
Postmenopausal				
Never used HT	56	13.9	30.6	
Past user of EPT	24	5.9	43.5	
Past user of ET	21	5.2	29.4	
Recent user of EPT	51	12.6	39.2	
Recent user of ET	43	10.6	34.8	
Unknown menopause	18	4.5	37.7	0.01
Age at mammogram (years)				
35–39	92	22.8	45.1	
40–44	84	20.8	41.2	
45–49	42	10.4	37.3	
50–54	76	18.8	34.0	
55–59	70	17.3	27.9	
60–64	40	9.9	26.7	<0.0001
Body mass index (kg/m^2)[d]				
<22	114	28.2	49.7	
22–24.9	108	26.8	38.2	
25–29.9	114	28.2	28.5	
≥30	68	16.8	20.5	<0.0001
Parity/age at first full-term pregnancy (years)[d]				
Nulliparous	85	21.0	44.3	
1–2/<24	80	19.8	34.0	
1–2/≥24	97	24.0	40.2	
≥3/<24	116	28.7	27.5	
≥3/≥24	26	6.4	32.1	<0.0001
Age at menarche (years)[d]				
<12	108	26.7	32.1	
12	108	26.7	35.4	
13	99	24.5	34.0	
>13	89	22.0	40.8	0.04
First-degree family history of breast cancer[d]				
No	326	80.7	35.2	
Yes	57	14.1	37.3	
Unknown	21	5.2	33.2	0.72

[a] Analysis of covariance.
[b] Adjusted for age at mammogram (years): 35–39, 40–44, 45–49, 50–54, 55–59, 60–64, and body mass index (kg/m^2): <22, 22–24.9, 25–29.9, 30+.
[c] HT, hormone therapy; EPT, estrogen progestin therapy; ET, estrogen therapy.
[d] Adjusted for age at mammogram.

Results

Our sample included 246 Caucasian and 158 African-American breast cancer patients, with mean age at the time of mammogram at 48.4 years (SD = 8.5). The mean percent mammographic density of African-American women (34.1%) and Caucasian women (34.3%) did not differ (Table 1). Mean percent density did vary substantially by age at mammogram, menopausal status and HT use, body mass index, pregnancy history, and age at menarche, but not by family history of breast cancer.

For all of the subjects, the number of *AR*-CAG repeats ranged from 10 to 35 (median, 21). Stratification by racial group indicated that the *AR*-CAG repeat distribution for African-Americans (range, 10–32; median, 20) was statistically significantly lower than that of Caucasians (range, 10–35; median, 22; $P < 0.0001$).

Mean percent mammographic density was compared across the three *AR* genotype categories, S/S, S/L, and L/L (Table 2). We observed no statistically significant associations between percent mammographic density and *AR* genotype in all of the subjects. On average, subjects with the L/L genotype had higher percent mammographic density (35.6%) than subjects with the S/S or S/L genotype (33.5%), but this difference was not statistically significant. There were also no statistically significant differences in mean percent mammographic density within subgroups defined by race, family history, or menopausal status.

We stratified postmenopausal women according to their histories of HT use (never HT, ever HT, and within the group who had used HT, according to whether their most recent use was ET or EPT). Among the EPT users, carriers of the L/L genotype had a statistically significant higher percent mammographic density (41.4%) than carriers of the S/S genotype (25.7%; $P = 0.04$). The results were comparable when this analysis was restricted to recent (within the last year) EPT users ($P = 0.03$). The results were also comparable when this analysis was restricted to Caucasians (68% of ever HT users; data not shown). Adjustment for other covariates, such as family history of breast cancer, parity, age at first full-term pregnancy, and age at menarche did not change the results ($P = 0.01$). In ET users, L/L carriers had a similar percent density (32.8%) to that of the S/S carriers (34.5%; $P = 0.83$).

In assessing the association between the *AR* genotype and a higher quartile in percentage mammographic density for each subgroup of HT usage (Table 3), we found that among women with a history of HT use, there was greater odds of having a single quartile higher level in percent density with each L allele; however, this was not statistically significant in the multivariate model (adjusted OR = 1.55; 95% CI:

Table 2 *Least-squares mean percentage breast density by AR[a] genotype*

Characteristic	*AR* Genotype S/S	S/L	L/L	L/L vs S/S	*P*	P_{trend}
All						
n	74	185	145			
%density[b]	34.8	33.0	35.6	0.79	0.50	0.64
Caucasians						
n	24	97	125			
%density[c]	36.2	33.6	35.3	0.84	0.78	0.86
African-Americans						
n	50	88	20			
%density[c]	35.4	33.9	39.8	0.40	0.48	0.62
Premenopausal						
n	35	90	66			
%density[b]	39.3	36.8	41.1	0.71	0.44	0.55
Postmenopausal						
n	34	85	76			
%density[b]	34.0	33.0	34.2	0.95	0.92	0.89
Ever HT						
n	22	65	52			
%density[b]	31.4	33.5	37.3	0.29	0.50	0.25
Never HT						
n	12	20	24			
%density[b]	38.2	33.6	26.0	0.11	0.27	0.11
ET users						
n	12	27	25			
%density[b]	34.5	31.6	32.8	0.83	0.92	0.86
EPT users						
n	10	38	27			
%density[b]	25.7	35.7	41.4	0.04	0.11	0.04
Current EPT user[d]						
n	9	32	22			
%density[b]	24.6	37.9	41.8	0.03	0.10	0.05

[a] AR, androgen receptor; EPT, estrogen progestin therapy; ET, estrogen therapy.
[b] Adjusted for race: African-American, Caucasian; age at mammogram (years): 35–39, 40–44, 45–49, 50–54, 55–59, 60–64; body mass index (kg/m^2): <22, 22–24.9, 25–29.9, ≥30.
[c] Adjusted for age at mammogram and body mass index.
[d] Current EPT=used EPT within 1 year before mammogram.

0.93–2.60, p trend = 0.10). In never HT users, there was a statistically significant protective effect of the L allele on increasing density; however, this was not observed in the generalized linear model or in the model adjusting for family history of breast cancer, parity, age at first full term pregnancy, and age at menarche. After stratifying by type of HT use we found a statistically significant higher odds for each L allele among EPT users [adjusted OR, 2.59; 95% confidence interval (CI), 1.20–5.60; P_{trend} = 0.02], but not among ET users (adjusted OR, 1.02; 95% CI, 0.46–2.25; P_{trend} = 0.97).

A formal test of interaction between EPT use and the *AR* genotype was not statistically significant on a multiplicative scale (P = 0.10).

Because no standard exists for classifying *AR*-CAG repeat lengths into S and L alleles, we categorized the *AR* genotype into 10 genotypes using the cut-points of 19, 21, and 24 to generate the alleles: very short (VS), medium short (MS), medium long (ML), and very long (VL). Sample sizes were small in the 10 strata; however, density was lowest in the VS/VS group of EPT users (13.0%) and increased as number of repeats increased up to the ML/ML group (43.2%), and then remained high in the ML/VL (39.9%) and VL/VL (56.4%) genotypes suggesting that the 21 repeat may be an appropriate threshold value.

Discussion

In this study the number of CAG repeats in the *AR* gene was not associated with mammographic density in all women combined or in subgroups based on race, menopausal status, or family history of breast cancer. The long CAG repeat (≥21 repeats) was strongly associated with increased percent mammographic density in postmenopausal women who were EPT users.

An increased breast cancer risk has been observed among women with the long CAG allele in three previous studies (3, 5, 7), whereas only a weak, nonsignificant association was observed in four studies (2, 4, 6, 8). The three positive studies were conducted among *BRCA1* mutation carriers (3), postmenopausal women (5), and women with a first-degree family history of breast cancer (7). Our observed association in EPT users is compatible with the study that found an association in postmenopausal women (5).

Mammographic density may have a strong genetic component. A study of twins reported significant higher correlation in mammo-

Table 3 *Association for a single quartile increase in percent mammographic density per "long" AR[a] allele*

AR genotype		% Density[b] <17	18–37	37–54	55+	Unadjusted OR	95% CI	P_{trend}	Adjusted[c] OR	95% CI	P_{trend}
Ever HT											
	SS	8	7	4	3						
	SL	15	23	17	10						
	LL	10	15	14	13	1.54	1.00–2.39	0.05	1.55	0.93–2.60	0.10
Never HT											
	SS	3	6	0	3						
	SL	11	3	5	1						
	LL	10	8	2	4	0.85	0.46–1.59	0.61	0.38	0.15–0.95	0.04
ET user											
	SS	5	3	2	2						
	SL	8	10	7	2						
	LL	7	7	7	4	1.30	0.71–2.40	0.39	1.02	0.46–2.25	0.97
EPT user											
	SS	3	4	2	1						
	SL	7	13	10	8						
	LL	3	8	7	9	1.85	0.98–3.46	0.06	2.59	1.20–5.60	0.02
Past EPT user[d]											
	SS	0	0	1	0						
	SL	0	3	1	2						
	LL	0	3	0	2	0.80	0.14–4.50	0.80	—	—	—
Current EPT user[d]											
	SS	3	4	1	1						
	SL	7	10	9	6						
	LL	3	5	7	7	2.05	1.03–4.06	0.04	—	—	—

[a] AR, androgen receptor; HT, hormone therapy; EPT, estrogen progestin therapy; ET, estrogen therapy; OR, odds ratio; CI, confidence interval.
[b] Frequency of subjects in each subgroup defined by AR genotype and percent mammographic density category.
[c] Adjusted for race: African-American, Caucasian; age at mammogram (years): 35–39, 40–44, 45–49, 50–54, 55–59, 60–64; body mass index (kg/m^2): <22, 22–24.9, 25–29.9, ≥30.
[d] Current EPT=used EPT within 1 year before mammogram.

graphic density between monozygotic twins than dizygotic twins (17). No data were presented on whether this association was stronger in twins who were both EPT users. Genes involved in estrogen metabolism have not shown to be associated with mammographic density in this study population (13). Using data from the Nurses' Health Study, Haiman *et al.* (9) observed no such association between mammographic density and the *AR* genotype. However, no analyses stratified by type of HT were reported in either of these studies.

Prior epidemiological studies have found that EPT is associated with higher percent mammographic density (18–23). In the placebo-controlled Postmenopausal Estrogen and Progesterone Intervention trial, women in the EPT treatment group underwent on average a 5% increase in mammographic density (23). However, the factors that determine the variation in the percent density response in the EPT arm of this trial are not known. Our results suggest that AR could be one such factor.

Ligands that bind AR with high affinity include not only testosterone and dihydrotestosterone, but also progestins (24). Human clinical data suggest that the AR may mediate the antiproliferative effects of high-dose synthetic progestins such as medroxyprogesterone acetate on breast cancer cells. In one study, the response rate to medroxyprogesterone acetate was significantly associated with the presence of AR ($P < 0.001$) with a shorter progression free interval in subjects with higher AR content (25). No data exist on how the much less-potent EPT, known to stimulate breast cell proliferation (26), affects AR.

Our analysis has some limitations that must be considered in interpreting results. First, we included a subset of breast cancer patients from the Women's CARE Study who had provided both a mammogram and a blood sample. This introduces the possibility of selection bias. For this to occur and explain the EPT findings, EPT using patients with long *AR*-CAG repeats and high mammographic density would have had to be more likely to have participated. This seems unlikely.

Second, we assessed the relationship between germ-line genotypes and mammographic density of breast cancer patients only. Although we used mammograms of the contralateral (unaffected) breast obtained before or at the time of diagnosis, these may not be comparable with mammograms from women without breast cancer. If there are other, unknown cofactors that are independent risk factors for breast cancer, and that also interact with the *AR*-CAG repeat length to increase mammographic density in EPT users, then our estimates of the impact of the *AR*-CAG repeat on mammographic density may not represent that of healthy women.

Third, a proportion of the heterozygotes for S/L may be misclassified. The *AR* gene is located on the X chromosome, and ~10% of breast cancer cases between the ages of 27 and 65 show preferential X-inactivation (27). Because only the S or the L allele is expressed in subjects that have one X chromosome inactivated, the heterozygote subjects with preferential X-inactivation would be more accurately grouped as a S/S or the L/L genotype. The effect of such misclassification would be a bias toward the null, and this may explain why some of our results for the S/L genotype are substantially weaker than those of the L/L genotype alone.

A final limitation in the analysis of the *AR* genotype is the method of dichotomizing the allele length into S *versus* L. The number of CAG repeats in the *AR* alleles ranged from 10 to 35 in our study. It has been observed that with increasing length, AR activity decreases (28). However, to our knowledge no threshold number of repeats has been reported. Our observation of a shorter repeat distribution in African-Americans is concordant with observations reported previously in studies of this polymorphism and prostate cancer (29, 30). In the absence of prior information on how to dichotomize allele length, we selected the median of the distribution of the *AR* repeat lengths as our cut-point. However, our sensitivity analysis using three successive cut-points supported the relevance of the 21 repeat cut-point.

Our results may help explain the mechanism by which EPT use increases breast cancer risk (31). Our results suggest that *AR* genotype modifies hormone-induced cell proliferation as reflected in percent mammographic density. Additional results are needed to determine whether knowledge of *AR* genotype will be helpful to clinicians in advising patients when making decisions on whether to use EPT.

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