Null Results in Brief

A Comprehensive Examination of *CYP19* Variation and Breast Density

Janet E. Olson,[1] Cynthia X. Ma,[7] Linda L. Pelleymounter,[3] Daniel J. Schaid,[4] V. Shane Pankratz,[4] Robert A. Vierkant,[4] Zachary S. Fredericksen,[4] James N. Ingle,[2] Yanhong Wu,[5] Fergus Couch,[6] Thomas A. Sellers,[8] Richard M. Weinshilboum,[3] and Celine M. Vachon[1]

[1]Division of Epidemiology, Department of Health Sciences Research; [2]Division of Medical Oncology, Department of Oncology; [3]Division of Clinical Pharmacology, Department of Molecular Pharmacology and Experimental Therapeutics; [4]Division of Biostatistics, Department of Health Sciences Research; [5]Genotyping Shared Resource; [6]Department of Laboratory Medicine and Pathology, Mayo Clinic College of Medicine, Rochester, Minnesota; [7]Division of Oncology, Siteman Comprehensive Cancer Center, Barnes-Jewish Hospital and Washington University School of Medicine, St. Louis, Missouri; and [8]Division of Cancer Prevention and Control, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida

Introduction

Mammographic density is one of the strongest risk factors for breast cancer. Numerous lines of evidence suggest that mammographic density is influenced by estrogen exposures (1-5). *CYP19* produces aromatase, the enzyme responsible for conversion of androgens to estrogens in all tissues, including the breast. This conversion of androgens to estrogens is important at all ages, especially after menopause when estrogen production takes place primarily in peripheral, nonovarian tissue (6). We report the results of comprehensive analyses of *CYP19* polymorphisms and percentage mammographic density (PD) in 550 healthy Caucasian women. We examined whether genetic variation, or haplotypes, in *CYP19* are associated with PD.

Materials and Methods

Study subjects were women without a history of cancer visiting Mayo Department of Internal Medicine for a medical exam. These women were controls for a breast cancer case-control study (described in ref. 7) who provided informed consent, a written questionnaire, and a blood sample. Postmenopausal status was defined as having no menstrual period for 12 months or having a hysterectomy or oophorectomy. Hormone therapy was categorized as ''currently taking,'' ''taken in the past,'' or ''never used.''

Mammograms (craniocaudal or top-down view of one breast) closest to the questionnaire date were digitized using a Kodak Lumiscan 85 scanner with 12-bit grayscale depth as described elsewhere (8). PD (dense area divided by total area $\times$ 100) was estimated using a computer-assisted thresholding program that has been routinely used in several studies (9). All images were read by a programmer, trained in the estimation of density, who has shown an intraobserver variation of less than 10%.

Polymorphisms common in Caucasian samples were selected from all variants identified through gene resequencing using two selection methods (10). The first method, which aims to tag common haplotypes (11), selected 12 variants (0.02 minimum minor allele frequency, 0.01 minimum haplotype frequency, 90% minimum haplotype R^2). The other method, which aims to tag common single nucleotide polymorphisms (SNP) correlated by linkage disequilibrium (12), also selected 12 SNPs (0.05 minor allele frequency, 80% correlation within bins). Six SNPs were chosen by both methods. Haplotype blocks within the gene were also identified (13). Genotyping was done as described previously (7). Genotyping quality was assessed by estimation of Hardy-Weinberg equilibrium and inclusion of 13 blinded duplicate samples. Most duplicates were concordant; however, the discrepancies that occurred resulted in at most two samples being discordant for any given SNP.

Single-variant analyses were done using multiple regression, and genotypes were modeled as having an additive relationship with PD. Analyses estimated the differences in PD associated with each additional copy of the variant allele. All models were adjusted for age and geographic region. Additional covariates that were significantly associated with PD in this sample were also included. PD values were skewed; therefore, they were square root transformed before analyses.

The associations between haplotypes and PD were assessed using a global test of association, followed by estimation of the linkage phase and the association of individual haplotypes with PD (14). Individual haplotype associations were considered statistically significant only if the global haplotype test was also significant.

Analyses were done in the SAS (SAS Institute, Cary, NC) and S-Plus (Insightful, Seattle, WA) statistical packages.

Results

Mammograms were available on 550 (75%) of the 732 women. Women without mammograms were younger and lived farther away from Mayo Clinic than those with mammograms. Otherwise, the groups were similar. Mean (untransformed) PD was inversely associated with age, number of children, and body mass index (BMI), and positively associated with duration of oral contraceptive use, alcohol intake, education level, and infertility (data not shown). With adjustment, only age, BMI, menopausal status, and parity/age at first birth remained significant.

Table 1 displays the PD estimates associated with each *CYP19* variant. None of the variants, including the nonsynonymous cSNPs (W39R, R264C, and T201M), were significantly

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Requests for reprints: Janet E. Olson, Mayo Clinic College of Medicine, 200 First Street Southwest, Rochester, MN 55901. Phone: 507-284-9833. E-mail: olsonj@mayo.edu

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Table 1. Association of PD (square root–transformed) with variants of CYP19A1 selected by haplotype-tagging methods of Carlson and/or Stram

CYP19 variant	Age, region, and BMI adjusted		Multivariate-adjusted*	
	Coefficient (SE)†	*P*‡	Coefficient (SE)†	*P*‡
5′FR exon 1.1 (−588)§	0.11 (0.16)	0.50	0.15 (0.16)	0.34
5′FR exon 1.1 (−144)‖	0.03 (0.16)	0.87	0.06 (0.16)	0.70
5′FR exon 2.a (−468)§,‖	0.05 (0.14)	0.71	0.08 (0.13)	0.55
5′FR exon 2.a (−429)‖	0.03 (0.25)	0.90	0.05 (0.24)	0.84
5′FR exon 1.5 (−628)§,‖	−0.02 (0.14)	0.86	0.01 (0.14)	0.92
Intron 1.7 (54)§	0.41 (0.23)	0.07	0.44 (0.24)	0.06
5′FR exon 1.f (−725)§	−0.21 (0.16)	0.19	−0.14 (0.16)	0.39
5′FR exon 1.2 (−596)§,‖	−0.03 (0.14)	0.81	0.01 (0.14)	0.93
Exon 1.2 (224)‖	0.05 (0.09)	0.58	0.01 (0.09)	0.93
5′FR exon 1.6 (−196)§,‖	−0.05 (0.10)	0.62	−0.02 (0.10)	0.85
Exon 1.6 (−77)‖	−0.00 (0.09)	0.99	−0.03 (0.09)	0.72
Intron 2 (−59)§,‖	−0.05 (0.09)	0.61	−0.01 (0.09)	0.89
Intron 4 (27)I/D§,‖	−0.15 (0.10)	0.15	−0.11 (0.10)	0.28
Intron 4 (VNTR)8¶	−0.11 (0.15)	0.49	−0.18 (0.15)	0.23
Intron 4 (VNTR)10¶	0.17 (0.42)	0.69	0.16 (0.41)	0.70
Intron 4 (VNTR)12¶	−0.41 (0.27)	0.13	−0.50 (0.26)	0.06
Intron 5 (602)§	0.04 (0.30)	0.89	0.14 (0.30)	0.63
Intron 7 (26)§	0.06 (0.18)	0.73	0.10 (0.18)	0.56
3′UTR Exon 10 (1531)‖	−0.03 (0.09)	0.77	0.02 (0.09)	0.80
3′UTR exon 10 (1673)§,‖	−0.13 (0.11)	0.23	−0.09 (0.11)	0.43

*Adjusted for age, geographic region, BMI, menopausal status, and parity/age at first birth.
†Genotypic regression coefficient, reflecting the estimated change in the square root of PD per extra copy of the minor allele, adjusted for all covariates in the model. For example, the −588 variant in exon 1.1 can be interpreted as follows: for each additional copy of the minor allele, there is a corresponding increase in the square root of PD of 0.11%.
‡Test for trend, linear regression analysis.
§Variants selected using the Carlson method.
‖Variants selected using the Stram method.
¶Relative to all other combined variants of intron 4 (VNTR)*n* not listed in the table.

associated with PD. Table 2 presents the two sets of haplotype analyses conducted using the variants selected by the two methods [htSNP (11) versus tagSNP (12)]. These analyses also showed no association with PD, either overall, or stratified by menopausal status, BMI, use of hormone therapy (current versus former/never), or among women age 40 years or more (data not shown). Finally, analyses of haplotype blocks within *CYP19* showed no association with PD (data not shown).

Discussion

Polymorphisms in aromatase (*CYP19*) have the potential to influence hormonally related traits, including PD. However, none of our analyses of individual variants or haplotypes showed an association between *CYP19* variants and PD. The other report of *CYP19* (including only two variants) and PD also found no association (15).

Tagging methods are an efficient way to select and genotype a maximally informative set of common variants within a candidate gene (11, 12). Results from the SNPs chosen by the two methods (11, 12) we used were similar. None of the variants or haplotypes selected by either method showed a significant association with density.

A strength of this study is the use of complete gene resequencing data to select the gene variants. Unlike the previous study, which examined only two *CYP19* variants with density, we investigated all common variation within this gene. A second strength of our study is our power: with the sample available and using a Bonferroni-corrected error rate of 0.0028 (dividing 0.05 by the 18 unique SNPs examined), our study had 80% power to detect differences of square root–transformed density as low as 0.36 and 0.76 (or 0.12 and 0.58 untransformed) for SNPs with minor allele frequencies of 0.3 and 0.1, respectively. Finally, we use a quantitative estimate of PD that has been shown to be strongly associated with breast cancer (16).

Limitations of this study include (*a*) potential for reduced generalizability due to the primarily Caucasian population; (*b*) including only 13 duplicate samples that may have resulted in undetectable genotyping errors that could have affected study power and resulted in bias toward null values; and (*c*) using women recruited as controls for another study. However, the density values in these women were similar to those seen previously (8).

In summary, we found no evidence that genetic variation in SNPs or haplotypes of *CYP19* was associated with PD in women.

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Table 2. The association of the haplotypes of variants selected with either the method of Stram (top) or Carlson (bottom) and increased breast density in women with high BMI

Hap no.	5′FR Exon 1.1 (−144) C>T	5′FR Exon 2a (−468) C>T	5′FR Exon 2a (−429) T>C	5′FR Exon I.5 (−628) G>C	Exon I.2 (−596) T>C	5′FR Exon I.2 (−224) G>C	5′FR Exon I.6 (−196) C>A	Exon I.6 (−77) C>T	Intron 2 (−59) C>T	Intron 4 (TTTA)n ‡,‖	3′UTR Exon 10 (1531) T>C	3′UTR Exon 10 (1673) G>T	Hap frequency*	Hap score†	P ‡,§
1	C	C	T	G	T	C	C	T	C	12	T	G	0.021	−1.329	0.184
2	C	C	T	G	T	C	C	T	C	8	T	G	0.087	−1.191	0.234
3	C	C	T	G	T	G	A	C	T	—	C	T	0.073	−1.141	0.254
4	C	C	T	G	T	G	C	C	T	—	C	T	0.024	−1.105	0.269
5	T	C	T	G	T	G	A	C	T	—	C	G	0.027	−1.035	0.301
6	C	C	T	C	C	G	A	C	T	—	C	T	0.088	−0.980	0.327
7	C	T	T	G	T	C	C	C	C	—	T	G	0.010	−0.930	0.352
8	C	T	T	G	T	C	C	T	C	12	T	G	0.010	−0.706	0.480
9	C	T	T	G	T	G	A	C	T	—	C	G	0.011	−0.452	0.651
10	T	C	T	G	T	G	C	C	T	—	C	T	0.014	−0.381	0.703
11	T	C	T	G	T	G	A	C	C	—	T	G	0.020	−0.059	0.953
12	C	C	T	G	T	C	C	T	C	—	T	G	0.167	0.001	0.999
13	C	C	T	G	T	C	C	C	C	8	T	G	0.012	0.018	0.986
14	C	C	C	G	T	C	C	T	C	—	T	G	0.029	0.404	0.686
15	C	C	T	G	T	C	C	C	C	—	T	G	0.025	0.759	0.448
16	C	C	T	G	T	G	A	C	T	—	C	G	0.106	0.773	0.439
17	C	T	T	G	T	C	C	T	C	—	T	G	0.077	0.951	0.342
18	C	T	T	G	T	G	C	C	T	—	C	T	0.041	1.517	0.129
19	C	C	T	C	C	G	A	C	T	—	C	G	0.036	1.801	0.072

Global-stat, 18.04; degrees of freedom, 19; $P = 0.52$§

Hap no.	5′FR Exon 1.1 (−588) G>A	5′FR Exon 2a (−468) C>T	5′FR Exon I.5 (−628) G>C	Intron 1.7 54 G>C	5′FR Exon I.f (−725) G>A	5′FR Exon 1.2 (−596) T>C	5′FR Exon 1.6 (−196) C>A	Intron 2 (−59) C>T	Intron 4 27 I>D	Exon 5 602 C>T	Intron 7 26 C>T	3′UTR 1673 G>T			
1	G	C	C	G	G	T	A	T	DEL	C	C	T	0.072	−1.514	0.13
2	G	C	G	G	G	T	C	T	INS	C	T	T	0.022	−1.352	0.176
3	G	C	G	G	A	C	A	T	DEL	C	C	T	0.081	−1.124	0.261
4	A	C	G	G	G	T	A	T	DEL	C	C	G	0.03	−0.831	0.406
5	G	C	G	G	G	T	C	C	INS	C	C	G	0.367	−0.52	0.603
6	A	C	G	G	G	T	C	T	INS	C	T	T	0.014	−0.349	0.727
7	G	T	G	G	G	T	C	C	INS	C	C	G	0.105	−0.227	0.82
8	A	C	G	G	G	T	A	C	INS	T	C	G	0.022	−0.006	0.995
9	G	C	G	G	G	T	A	T	DEL	C	C	G	0.11	−0.809	0.418
10	G	T	G	G	G	T	C	T	INS	C	T	T	0.045	1.36	0.174
11	G	C	C	C	G	C	A	T	INS	C	C	G	0.037	1.509	0.131
12	A	C	G	G	G	T	C	C	INS	C	C	G	0.021	2.31	0.0205

Global-stat, 17.92; degrees of freedom, 12; $P = 0.12$§

NOTE: Associations are adjusted for age, geographic region of residence, menopausal status, and parity/age at first birth. Stram method: 2% minor frequency, 1% minimum haplotype frequency, 90% correlation within bins. Carlson method: variants were required to have at least a 5% frequency and 80% correlation within bins. Abbreviations: Hap, haplotype; DEL, deletion; INS, insertion; FR, Flanking Region; UTR, Untranslated Region.

*Estimated haplotype frequency.

†Score statistic comparing the haplotype of interest to all other haplotypes combined. Negative values imply decreased PD; positive values imply increased density.

‡P value comparing the haplotype of interest with all other haplotypes combined.

§Individual haplotypes with $P < 0.05$ are not considered statistically significant unless the global P value is also <0.05.

‖Alleles are those never reported in the scientific literature to be associated with risk of breast cancer (TTTA)n, n = 7, 9, 11, or 13 versus n = 8, 10, and 12.

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