Research Article

Cancer Epidemiology, Biomarkers & Prevention

Associations between Polymorphisms in Glucuronidation and Sulfation Enzymes and Mammographic Breast Density in Premenopausal Women in the United States

Mellissa Yong[1,2], Stephen M. Schwartz[1,2], Charlotte Atkinson[4], Karen W. Makar[1], Sushma S. Thomas[1], Katherine M. Newton[2,3], Erin J. Aiello Bowles[3], Victoria L. Holt[1,2], Wendy M. Leisenring[1], and Johanna W. Lampe[1,2]

Abstract

Objective: Sex hormones are metabolized to less active compounds via (*a*) glucuronidation catalyzed by UDP-glucuronosyltransferases (UGT) and (*b*) sulfation catalyzed by sulfotransferases (SULT). Functional UGT and SULT polymorphisms can affect clearance of sex hormones, thereby influencing exposure in hormone-sensitive tissues, such as the breast. We assessed relationships between functional polymorphisms in the UGT and SULT genes and breast density in premenopausal women.

Methods: One hundred seventy-five women ages 40 to 45 years, who had a screening mammogram taken within the previous year, provided a genomic DNA sample. Mammograms were digitized to obtain breast density measures. Using generalized linear regression, we assessed associations between percent breast density and polymorphisms in the *UGT1A* and *UGT2B* families, *SULT1A1*, and *SULT1E1*.

Results: Women with the *SULT1A1(H213/H213)* genotype had 16% lower percent breast density compared with women with the *SULT1A1(R213/R213)* genotype after controlling for ethnicity ($P = 0.001$). Breast density was 5% lower among women carrying at least one copy of the *UGT1A1(TA7)-UGT1A3(R11)-UGT1A3(A47)* haplotype compared with the *UGT1A1(TA6)-UGT1A3(W11R)-UGT1A3(V47A)* haplotype ($P = 0.07$). No associations were observed between polymorphisms in the *UGT2B* family or *SULT1E1* and breast density.

Conclusion: Polymorphisms in *SULT1A1* and the *UGT1A* locus may influence percent breast density in premenopausal women. *Cancer Epidemiol Biomarkers Prev; 19(2); 537–46. ©2010 AACR.*

Introduction

Breast cancer is the most common cancer among women, with a lifetime probability of 1 in 8 in the United States (1). Aside from family history, the most well-established risk factors for breast cancer are those associated with hormonal and reproductive factors that result in greater lifetime exposure to estrogens and androgens (2), such as an extended reproductive life (resulting from an early age at menarche and late age at menopause), late age at first full-term pregnancy, and nulliparity. These observations, along with the finding that higher plasma concentrations of total and free estradiol (E_2) in the early follicular phase and total and free testosterone in both menstrual cycle phases are associated with an increased risk of breast cancer in premenopausal women (3), suggest that cumulative estrogen exposure is sufficient to alter breast cancer risk later in life.

Breast density may reflect lifelong hormone exposure and potentially could be used as a biomarker for breast cancer risk. Several studies have shown an inverse association between parity and mammographic density (4). Nulliparous women and women with a later age at first birth have higher estrogen levels than parous women and women with a younger age at first birth, respectively (5). Nulliparous women have denser breast tissue than parous women, and density decreases with increasing number of children (6). Moreover, among parous women, later age at first birth and fewer live births have been associated with a higher proportion of dense breast tissue and greater risk for breast cancer (6).

Glucuronidation catalyzed by UDP-glucuronosyltransferases (UGT; ref. 7) and sulfation catalyzed by sulfotransferases (SULT; ref. 8) are two pathways through which sex hormones are metabolized to less active compounds. Polymorphisms that alter enzyme function have been identified in UGT and SULT genes, and these may ultimately affect the clearance of, and therefore exposure to, endogenous and exogenous estrogens and androgens. Thus, individual variation in estrogen and androgen metabolism resulting from common genetic polymorphisms

Authors' Affiliations: [1]Fred Hutchinson Cancer Research Center; [2]Department of Epidemiology, University of Washington; [3]Group Health Research Institute, Seattle, Washington and [4]Department of Oral and Dental Science, University of Bristol, Bristol, United Kingdom

Corresponding Author: Johanna W. Lampe, Cancer Prevention Program, Fred Hutchinson Cancer Research Center, P.O. Box 19024, M4-B402, Seattle, WA 98109. E-mail: jlampe@fhcrc.org

doi: 10.1158/1055-9965.EPI-09-0898

could be a risk factor for hormone-dependent diseases and may serve as genetic markers of differences in lifetime hormonal exposure. One approach to determine whether these hormone-metabolizing gene polymorphisms affect cumulative exposure to estrogens and androgens throughout life is to assess their relationship to mammographic breast density.

In a study population of premenopausal women, we assessed the associations of selected functional polymorphisms in the *UGT1A* and *UGT2B* gene families and *SULT1A1* with mammographic breast density. We hypothesized that alleles with increased conjugating activity resulting in increased clearance of endogenous hormones and lower circulating hormone concentrations [*UGT1A3(R11)*, *UGT1A3(A47)*, *UGT2B7(Y268)*, and *UGT2B15(D85Y)*] would be associated with decreased breast density (via lower lifelong hormone exposure). Likewise, alleles with decreased conjugating activity resulting in decreased clearance of endogenous hormones and higher circulating hormone concentrations [*UGT1A1(TA7)*, *UGT2B17(null)*, and *SULT1A1(H213)*] would be associated with increased breast density (via higher lifelong hormone exposure).

Materials and Methods

Study Population

As described in detail elsewhere (9), women were recruited from within Group Health, a large integrated health plan in Washington State. Premenopausal women ages 40 to 45 years who have undergone a screening mammogram in the previous 10 months and who were not taking exogenous hormones were identified from the Group Health Breast Cancer Screening Program and recruited based on the Breast Imaging Reporting and Data System density score assigned to their most recent screening mammogram.

A total of 203 women attended a study clinic visit. At the time of consent, we asked each participant to indicate whether she was willing to have her stored biological samples used for future studies. A total of 189 (93%) study participants checked "yes" to this question, of whom 176 (93%) had a buffy coat available for genotyping. The major reason for those who did not have a buffy coat available was a problematic blood draw that resulted in no blood sample. We excluded one participant with a mammogram that was too dark to read, leaving a total of 175 women in our analyses. All study procedures were approved by the institutional review boards of the Fred Hutchinson Cancer Research Center and Group Health, and all study participants provided written informed consent.

Mammographic Breast Density Data

Each participant's most recent routine Group Health X-ray screening mammogram before her study visit was digitized using a Lumysis 85 scanner. A single reader interpreted films using Cumulus for percent density, dense area size, and total area size as described in detail elsewhere (10).

Genotyping of *UGT1A*, *UGT2B*, *SULT1A1*, and *SULT1E1* Polymorphisms

DNA for genotyping was extracted from the buffy coat fraction using the Qiagen blood kit. The concentration and purity were determined by spectroscopy at 260 and 280 nm. A total of 11 polymorphisms were genotyped (*UGT1A1(TA6/TA7)*, *UGT1A3(W11R)*, *UGT1A3(V47A)*, *UGT2B4(D458E)*, *UGT2B7(H268Y)*, *UGT2B15(D85Y)*, *UGT2B17(null/not null)*, *SULT1A1(R213H)*, *SULT1E1(I169A>G)*, *SULT1E1[I1(-73)G>C]*, and *SULT1E1[I5(-10)C>G]*) using a variety of PCR-based methods, including size-dependent separation, restriction fragment length polymorphism, sequencing, and fluorescent allelic discrimination (TaqMan). For *SULT1E1*, we selected three single nucleotide polymorphisms (*SULT1E1(I169A>G)*, *SULT1E1[I1(-73)G>C]*, and *SULT1E1[I5(-10)C>G]*) that were found by Adjei et al., which were used to distinguish the most common haplotypes (>5% allele frequency) in a Caucasian American population (11). Primers and probes for each polymorphism are shown in Table 1. Negative controls (no DNA template) and positive controls (cell line DNA and/or DNA samples of known genotypes) were run on every plate. The reliability/reproducibility of the genotyping assays was assessed by randomly selecting and re-assaying 5% of the samples for each run; no discrepancies were observed between initial and duplicate assays. Genotype calling was done both by machine and by one reader. If there was a discrepancy between the two calls, then an independent reader was brought in to resolve the difference. Samples for which we obtained an ambiguous result or did not obtain a genotyping result were repeated. Definitive results for repeated samples were obtained on the second attempt. Thus, we obtained genotyping results for the polymorphisms for all study participants.

UGT1A1(TA6/TA7)

Genotyping of the *UGT1A1(TA6/TA7)* (rs8175347) polymorphism was done as described previously (12). The fragments were analyzed using an ABI PRISM 3100 Genetic Analyzer and Genotyper 2.5 software (Applied Biosystems).

UGT1A3(W11R) and *UGT1A3(V47A)*

Genotyping of the two-residue amino acid substitutions *W11R* (rs3821242) and *V47A* (rs6431625) of *UGT1A3* involved two steps (13). First, PCRs were done to amplify the region of interest. Second, the PCR amplicons were sequenced. The sequence data were analyzed using the Sequencher 4.1 (Gene Codes) software.

UGT2B4(D458E)

A difference of one nucleotide in *UGT2B4* leads to a single amino acid change of aspartic acid to glutamic acid

at position 458. PCR was used to amplify the fragment containing *D458E* (rs13119049) as described previously (14). We then performed restriction fragment length polymorphism using *Taq*I restriction enzyme on the PCR product and separated the fragments on a 2% NuSieve gel. The expected fragment sizes for the *D458* allele were 232 and 32 bp, and the expected fragment size for the *E458* allele was 264 bp.

Table 1. Primer and/or probe sequences for genotyping

Polymorphism	Sequence
UGT1A1	
PCR primers	
FP	6FAM-GTCACGTGACACAGTCAAAC-3′
RP	5′-GTTTCTTTTTGCTCCTGCCAGAGGTT-3′
UGT1A3	
PCR primers	
FP	5′-AGTGAGCACAGGGTCAGACGT-3′
RP	5′-TCCAGGATGGATCAGTTCCA-3′
Sequencing primers	
FP1	5′-GCTCAGTGACAAGGTAATTA-3′
RP1	5′-GAAGGCTATTATGACAAGGA-3′
FP2	5′-CACACTCAACTGTACTTTGAA-3′
RP2	5′-CTTTGCATGAATGTCATGT-3′
UGT2B4	
PCR primers	
FP	5′-TTCATCATGATCAACCAGTGA-3′
RP	5′-CTTCCAGCCTCAGACGTAAT-3′
UGT2B7	
PCR primers	
FP	5′-GGCTTATTCGAAACTCCTGGAA-3′
RP	5′-TGGAGTCCTCCAACAAAATCAA-3′
Probes	
C	6FAM-AGTTTCCAcATCCAC-MGBNFQ
T	VIC-TTTCCAtATCCACTCTT-MGBNFQ
UGT2B15	
PCR primers	
FP	5′-GCCAGTAAATCATCTGCTATTAAATTAGAA-3′
RP	5′-GCATCTTTACAGAGCTTGTTACTGTAGTCATA-3′
Probes	
T	6FAM-TCAGAAGAGAATCTTCCAAATAATTT-MGBNFQ
G	VIC-TCAGAAGAGAATCTTCCAAATCATTT-MGBNFQ
UGT2B17	
PCR primers	
Exon1 FP	5′-TGAAAATGTTCGATAGATGGACATATAGTA-3′
Exon1 RP	5′-GACATCAAATTTTGACTCTTGTAGTTTTC-3′
Deletion FP	5′-TTTAATGTTTTCTGCCTTATGCCAC-3′
Deletion RP	5′-AGCCTATGCAATTTTCATTCAACATAG-3′
Probes	
Exon1	6FAM-TACATTTTGGTCATATTTTTCACAACTACAAGAATTGT-MGBNFQ
Deletion	JOE-ACTACACTGAGATTTACAAAAGAATTCTGTCAGGATATAG-MGBNFQ
SULT1A1	
PCR primers	
FP	5′-AGTTGGCTCTGCAGGGTTTCT-3′
RP	5′-ACCACGAAGTCCACGGTCTC-3′
Probes	
R	VIC-TGGCAGGGAGCGC-MGBNFQ
H	6FAM-CTGGCAGGGAGTGC-MGBNFQ

Genotyping of *UGT2B7, UGT2B15, UGT2B17, SULT1A1,* and *SULT1E1* Polymorphisms

We genotyped the polymorphisms in *UGT2B7* (rs7439366), *UGT2B15* (rs1902023), *SULT1A1* (rs9282861; ref. 15), and *SULT1E1* (rs3775768, rs4149530, and rs1220702; ref. 11) and a deletion in *UGT2B17* (16) using TaqMan. Data were analyzed with SDS software (Applied Biosystems), and genotype calls were based on the level of fluorescence emission from the reporter dye. With the exception of *UGT2B7* and *UGT2B15*, assays for the selected genotypes have been validated previously. Validation of the *UGT2B7* and *UGT2B15* assays was done using DNA samples (n = 20-100) from other studies that have been genotyped previously by restriction fragment length polymorphism and sequencing with no discrepancies between the results obtained from the two assays.

Data Analysis

We assessed the genotypes at each locus for consistency with the Hardy-Weinberg equilibrium using a χ^2 test. Measures of central tendency and categorical distributions were calculated to describe the characteristics of the study population, and initial assessments were done using non-model-based approaches including simple means and t tests.

Strong linkage disequilibrium was observed between *UGT1A3(W11R)* and *UGT1A3(V47A)* polymorphisms ($D' = 0.98$) and between *UGT1A1(TA7)* and *UGT1A3(W11R)* ($D' = -0.96$) and *UGT1A1(TA7)* and *UGT1A3(V47A)* ($D' = 0.98$) polymorphisms. Because the functional effect of *UGT1A1(TA7)* decreased *UGT1A1* gene expression and therefore decreased glucuronidation and *UGT1A3(W11R)* and *UGT1A3(V47A)* results in increased glucuronidation, we inferred haplotypes involving these three loci for our study population. For each gene (*UGT1A* and *SULT1E1*), we performed a global test of all the haplotypes versus no haplotypes using a likelihood ratio test for mammographic breast density. We then fit a generalized linear model with additive haplotype effects under Hardy-Weinberg equilibrium to test for an association between each of the inferred *UGT1A1-UGT1A3* and *SULT1E1* haplotypes and mammographic breast density (17).

To extend results from Sillanpää et al., who reported an inverse association between *SULT1A1(H213)* alleles and breast cancer among premenopausal women with high parity only (18), we also explored the effect of number of pregnancies (0, 1-2, and ≥3) on the association between *SULT1A1* genotypes and mammographic breast density. We used the *SULT1A1(R213/R213)* genotype as the reference group for each live birth category (0, 1-2, and ≥3) to compute the mean percent density values for the *SULT1A1(R213/H213)* and *SULT1A1(H213/H213)* genotypes. We performed a test for interaction between number of live births and *SULT1A1* genotype using a likelihood ratio test, which tests the full model (contains the interaction) against the reduced model (no interaction).

Genotypes were coded on an ordinal scale [homozygous wild-type (wt/wt) = 0, heterozygous (wt/v) = 1, and homozygous variant (v/v) = 2] to model allele dosage effects with the wt/wt genotype as the reference category. Genotype was also examined by using a dichotomous variable to indicate whether the participant was a carrier (wt/v or v/v) of the variant allele if no gene dosage effect was observed. Adjusted generalized linear regression models were fit to determine mean percent breast density by genotype, and a test for trend was conducted between the ordinal genotype measures and breast density measures using adjusted linear regression.

Previous studies examining race/ethnicity and UGT and SULT genotypes have reported race/ethnicity to be associated with genotypes (14, 19). For example, Lampe et al. (12) showed that both allele and genotype frequencies of *UGT1A1(TA6/TA7)* varied by race (White versus Asian). Race/ethnicity has also been shown to be associated with mammographic breast density with the highest mean percent density reported for African American women and the lowest reported for Japanese women (20). Thus, ethnicity (categorized: Asian, White, Other) was included in our final models.

Mean percent breast density and 95% confidence intervals are presented. Data were analyzed using Stata/SE (version 9.0; StataCorp), and haplotypes were inferred using Hapstat (software for the statistical analysis of haplotype-disease association; Copyright © 2006-2008 Tammy Bailey, Danyu Lin, and the University of North Carolina at Chapel Hill). A two-sided $P < 0.05$ was considered statistically significant.

Results

The mean (SD) age of the study participants was 42.4 (1.4) years, and the majority had one or more live births, had a history of hormone use (e.g., oral contraceptives, hormone patches, hormone injections, hormone implants, and intrauterine devices containing progesterone), and were nonsmokers (never or former), White, and highly educated (Table 2). The haplotype frequencies of *UGT1A* and *SULT1E1*, which satisfied Hardy-Weinberg equilibrium, and all genotype frequencies with nonsignificant χ^2 tests at $P > 0.05$ are presented in Table 3.

After adjusting for ethnicity, women with *UGT1A3 (W11/R11)* and *UGT1A3(R11/R11)* genotypes had lower mean percent mammographic breast density compared with women with the wild-type genotype [*UGT1A3 (W11/W11)*; 35.1% and 31.6%, respectively, versus 40.9%; $P_{trend} = 0.04$; Table 3]. There was a nonstatistically significant inverse association between *UGT1A1(TA7)-UGT1A3(R11)-UGT1A3(A47)* haplotype and mammographic breast density compared with the more common *UGT1A1(TA6)-UGT1A3(W11R)-UGT1A3(V47A)* haplotype in this population (35.7% versus 40.6%, respectively; $P = 0.07$; Table 3). Mean mammographic breast densities for women with the *SULT1A1(R213/H213)* and *SULT1A1 (H213/H213)* genotypes were lower compared with

Table 2. Characteristics of premenopausal women in the study population: Group Health, Seattle, WA, 2004-2005 (n = 175)

	n (%)
Age, y	
Mean (SD)	42.4 (1.4)
Median (range)	43.0 (40-45)
Age at menarche, y	
Mean (SD)	12.8 (1.3)
Median (range)	13.0 (10-17)
Age at first birth*, y	
Mean (SD)	28.8 (5.9)
Median (range)	29.0 (15-40)
Body mass index, kg/m^2	
Mean (SD)	25.8 (4.6)
Median (range)	25.0 (19-39)
Height, m	
Mean (SD)	1.65 (0.07)
Median (range)	1.65 (1.48-1.84)
Weight, kg	
Mean (SD)	70.4 (13.3)
Median (range)	68.0 (46-108)
Waist-to-hip ratio	
Mean (SD)	0.79 (0.06)
Median (range)	0.78 (0.66-1.00)
No. live births	
0	50 (28.6)
1	23 (13.1)
≥2	83 (47.4)
Had a history of breast-feeding*	100 (81.3)
Had a history of hormone use†	125 (71.4)
First degree relative with breast and/or ovarian cancer	22 (12.6)
Smoking status	
Current	8 (4.6)
Former	54 (30.9)
Never	119 (68.0)
Race/ethnicity	
Asian	13 (7.4)
White	152 (86.9)
Other	8 (4.6)
Years of school completed	
<12	12 (6.9)
13-15	48 (27.4)
16	49 (28.0)
>17	64 (36.6)
Income	
<$49,999	28 (16.0)
$50,000-75,000	41 (23.4)
>$75,000	83 (47.4)
No information provided	21 (12.0)

NOTE: Numbers (%) may not add up to 175 (100%) for some characteristics due to missing values and rounding calculations.

*Among parous women only (n = 123).

†Use of oral contraceptives, hormone patches, hormone injections, hormone implants, or intrauterine devices containing progesterone at any time before the 6-month period before the screening mammogram.

women homozygous for *SULT1A1(R213)* (34.9% and 25.7% versus 41.8%, respectively; P_{trend} = 0.001; Table 3). We also observed nonsignificant inverse associations between both the *TA7* allele of *UGT1A1* and the *Y268* allele of *UGT2B7* and mammographic density and a nonsignificant positive association between the *E458* allele of *UGT2B4* and mammographic density (Table 3).

For the polymorphisms for which we did not observe a dose-response relationship, a borderline statistically significant inverse association was shown between percent mammographic breast density and *UGT1A3(V47A)*, with carriers of the *A47* allele having a 6.9% lower percent density compared with noncarriers [percent density (95% confidence interval), 33.6% (29.3-37.8%) for carriers and 40.4% (35.0-45.8%) for noncarriers; P = 0.050; data not shown]. No statistically significant associations between percent mammographic breast density and either *UGT2B15(D85Y)* or *UGT2B17(deletion)* were observed.

Of the three *SULT1E1* single nucleotide polymorphisms genotyped, one (rs1220702) did not occur as frequently relative to the other two (minor allele frequency = 11%) and did not contribute to the delineation of any common haplotype. Among the two remaining *SULT1E1* single nucleotide polymorphisms, we identified three haplotypes in our study population. No significant association was shown between mammographic breast density and the *SULT1E1* haplotypes. The likelihood ratio test comparing the model with the haplotype effects of *SULT1E1* (full model) to the model with no haplotypes (reduced model) showed that the model without the haplotypes provided an adequate fit to the data.

We assessed the interaction of *SULT1A1* genotypes and number of live births on mammographic breast density. Mammographic density decreased with increasing number of *H213* alleles within each category of live births (0, 1-2, and ≥3 live births). The reduction in percent density between the *R213/H213* and *H213/H213* genotypes and the reference genotype (*R213/R213*) was more pronounced in women who had no live births [absolute differences: −8.0% (*R213/H213*) and −18.9% (*H213/H213*);

P_{trend} = 0.049] compared with women with 1-2 and ≥3 live births (Table 4). However, no statistically significant interaction between *SULT1A1* genotypes and number of live births was shown (χ^2 = 1.48; P = 0.83).

Discussion

In this well-characterized population of healthy, premenopausal women, we assessed the associations

Table 3. Adjusted mean percent mammographic density of study population by genotype and haplotype: Group Health, Seattle, WA, 2004-2005

Genotype	*n* (%)	Mean* (95% confidence interval)	*P*	P_{trend}
*UGT1A1*28*				
TA6/TA6	91 (52.0)	39.0 (34.0-44.1)	Reference	0.12
TA6/TA7	69 (39.4)	35.0 (30.0-39.9)	0.26	
TA7/TA7	15 (8.6)	30.7 (19.7-41.7)	0.18	
UGT1A3(W11R)				
W11/W11	68 (38.9)	40.9 (35.0-46.9)	Reference	0.04
W11/R11	74 (42.3)	35.1 (30.3-40.0)	0.14	
R11/R11	33 (18.9)	31.6 (24.3-38.9)	0.05	
UGT1A3(V47A)				
V47/V47	80 (45.7)	40.4 (35.0-45.8)	Reference	0.11
V47/A47	71 (40.6)	33.2 (28.4-37.9)	0.05	
A47/A47	24 (13.7)	34.8 (25.6-44.1)	0.30	
UGT2B4(D458E)				
D458/D458	97 (55.4)	35.0 (30.2-39.8)	Reference	0.25
D458/E458	68 (38.9)	38.2 (33.1-43.4)	0.37	
E458/E458	10 (5.7)	42.6 (27.0-58.3)	0.36	
UGT2B7(H268Y)				
H268/H268	48 (27.4)	40.4 (33.6-47.3)	Reference	0.22
H268/Y268	86 (49.1)	35.8 (31.0-40.7)	0.29	
Y268/Y268	41 (23.4)	34.3 (27.3-41.4)	0.23	
UGT2B15(D85Y)				
D85/D85	35 (20.0)	39.4 (33.1-45.7)	Reference	0.50
D85/Y85	94 (53.7)	36.2 (31.6-40.8)	0.41	
Y85/Y85	46 (26.3)	35.7 (27.9-43.5)	0.47	
UGT2B17(deletion)				
not deleted/not deleted	73 (41.7)	38.1 (32.6-43.6)	Reference	0.80
not deleted/deleted	75 (42.9)	34.3 (29.3-39.3)	0.31	
deleted/deleted	27 (15.4)	39.6 (29.7-49.5)	0.80	
SULT1A1(R213H)				
R213/R213	83 (47.4)	41.8 (36.5-47.1)	Reference	0.001
R213/H213	64 (36.6)	34.9 (29.6-40.2)	0.08	
H213/H213	28 (16.0)	25.7 (18.4-33.0)	0.001	
Haplotype	**Frequency**			
UGT1A1(TA6/TA7)-UGT1A3(W11R)-UGT1A3(V47A)				
TA6-W11-V47	0.59	40.6 (33.9-47.4)	Reference	N/A
TA6-R11-V47	0.06	32.4 (15.6-49.4)	0.12	
TA6-R11-A47	0.06	38.4 (22.5-54.7)	0.66	
TA_7-R11-A47	0.28	35.7 (23.8-47.8)	0.07	
SULT1E1: rs3775768 (A/G) and *rs4149530 (G/C)*				
A-G	0.57	37.6 (31.1-44.2)	Reference	N/A
A-C	0.05	38.5 (18.5-59.0)	0.87	
G-C	0.26	35.4 (23.9-47.5)	0.47	

*Adjusted for ethnicity using least squares regression. N/A, not applicable.

Table 4. Association between *SULT1A1* genotypes and mammographic breast density of study population by number of pregnancies: Group Health, Seattle, WA, 2004-2005

No. live births	*SULT1A1* genotype	*n** (%)	Mean† (95% confidence interval)	Absolute % difference	*P*	P_{trend}
0	*R213/R213*	26 (16.7)	49.3 (40.3-58.4)	Reference	—	0.05
	R213/H213	16 (10.2)	41.3 (32.8-49.9)	–8.0	0.21	
	H213/H213	8 (5.1)	30.4 (12.5-48.3)	–18.9	0.07	
1-2	*R213/R213*	38 (24.4)	38.6 (31.1-46.1)	Reference	—	0.05
	R213/H213	33 (21.2)	33.6 (25.7-41.4)	–5.0	0.38	
	H213/H213	13 (8.3)	25.1 (15.4-34.8)	–13.4	0.04	
≥3	*R213/R213*	9 (5.8)	31.3 (13.0-49.6)	Reference	—	0.30
	R213/H213	7 (4.5)	23.4 (6.7-40.1)	–7.9	0.52	
	H213/H213	6 (3.8)	19.1 (3.6-34.7)	–12.2	0.31	

NOTE: $P_{interaction}$ = 0.83.
*Numbers do not add up to 175 because 19 participants were missing data on number of live births.
†Adjusted for ethnicity using least squares regression.

between percent mammographic breast density and polymorphisms in the *UGT1A, UGT2B, SULT1A1*, and *SULT1E1* genes. We observed a strong significant inverse association between percent mammographic breast density and *SULT1A1(H213/H213)* carriership. This finding is counterintuitive given that the protein coded by *SULT1A1(H213/H213)* has been shown to be associated with lower enzyme thermostability, lower enzyme activity, and lower capacity to sulfate E_2 and catechol estrogens compared with the wild-type [*SULT1A1(R213/R213)*; ref. 21]. Our results could, however, reflect the role of catechol estrogens (2- and 16α-hydroxyestrone). 2-Hydroxyestrone is conjugated to anticarcinogenic methoxylated metabolites (e.g., 2-methoxyestrone and 2-methoxyestradiol) and is hypothesized to protect against breast cancer (22). In contrast, 16α-hydroxyestrone is a potent estrogen, has been shown to form covalent bonds with estrogen receptors, and appears to be genotoxic (23). Findings reported by others (24, 25) suggest that these catechol estrogen metabolites may be involved in the etiology of breast cancer and this effect may be mediated, in part, by percent breast density. Hui et al. (26) recently showed that human SULT enzymes are capable of sulfating catechol estrogens and methoxyestrogens in breast cancer cells and human mammary epithelial cells. Therefore, it is possible that the lower conjugating activity of *SULT1A1(H213/H213)* might increase the availability of estrogens for conversion to catechol estrogens and subsequent conjugation to methoxyestrogens. In addition, given that *SULT1A1* has been shown to be an efficient and selective catalyst of 2-methoxyestradiol sulfation (27), it is possible that *SULT1A1* could modify the effects of 2-methoxyestradiol. Women with low activity *SULT1A1(H213/H213)* genotype could have higher levels of the unconjugated form of 2-methoxyestradiol. Consequently, the potential protective effects of this metabolite may be prolonged in women with low sulfation capacity compared with women with high sulfation activity.

Based on our findings of lower mammographic breast density with increasing numbers of the *H213* allele, we might expect premenopausal women with the *H213* allele to have a decreased risk of breast cancer. Two studies have examined the association between *SULT1A1* genotypes and breast cancer risk in premenopausal women and neither found a significant genotype effect on overall breast cancer risk (18, 28). However, Sillanpää et al. showed an inverse association of this allele with breast cancer in premenopausal women with high parity, suggesting a modifying effect of full-term pregnancies (18). In contrast, our findings suggested that the inverse association between the *H213* allele and breast density was most pronounced in women with no pregnancies, and the inverse trend became weaker as the number of live births increased; however, our finding was not statistically significant and our study was underpowered for the analysis of this interaction (power = 0.093). Nonetheless, if confirmed in other studies, our results suggest that the effect of this polymorphism may be strongest when the substrate is highest, given that low parity results in higher lifelong estrogen exposure, whereas higher parity results in lower lifelong estrogen exposure.

We did not observe significant associations between mammographic breast density and polymorphisms in *UGT2B15* and *UGT2B17*, although there was a nonsignificant inverse trend with the *UGT2B15(D85Y)* allele in the hypothesized direction. We also did not observe an association between mammographic breast density and common *SULT1E1* haplotypes. It is possible that the single nucleotide polymorphisms identified by Adjei et al. (11) may not have any functional consequences for steroid hormone sulfation or that there is a true effect but we did not have the statistical power to detect it in our study.

In vitro studies of the *TA7* allele have reported a 30% reduction in *UGT1A1* gene transcription and decreased *UGT1A1* gene expression (29-31), and individuals deficient in *UGT1A1* due to a deletion encompassing the promoter and first exon exhibit a 70% decrease in the glucuronidation of E_2 (32). Thus, we hypothesized that carriers of the *TA7* allele should have higher lifelong estrogen exposure and higher risks of estrogen-related conditions, including increased mammographic density. Our finding and that of Haiman et al. (33) that percent breast density was 8% and 16% lower, respectively, in premenopausal women with the *UGT1A1(TA7/TA7)* genotype compared with those with the *UGT1A1(TA6/TA6)* genotype appear to contradict this hypothesis. However, strong linkage disequilibrium exists between the *UGT1A1(TA7)* allele and apparently functional polymorphisms in multiple other *UGT1A* family genes [including *UGT1A6* (12, 34), *UGT1A3* (13), and *UGT1A7* (35)]. To date, no studies have looked at associations between estrogen glucuronidation and *UGT1A* haplotypes. Although not statistically significant, we found that mean percent breast density was lowest for women who carry at least one copy of the *UGT1A1(TA6)-UGT1A3(R11)-UGT1A3(V47A)* haplotype.

This is consistent with our observation of a statistically significant inverse association between mammographic breast density and the *UGT1A3(R11)* allele. The difference in breast density that we observed according to *UGT1A3* genotype is consistent with the greater clearance of estrogen expected in women with the *R11* allele. However, the *R11* allele has little effect on mammographic density in combination with the *UGT1A3(A47)* allele and only a small effect when inherited with the *UGT1A1(TA7)* allele. These findings suggest that, at the *UGT1A* locus, the coinheritance of *UGT1A3(R11)* and *UGT1A3(V47A)*, or other variation on the haplotype containing these alleles, has the strongest influence on mammographic density. Thus, the reduced mammographic density that we and Haiman et al. have observed associated with the *UGT1A1(TA7)* allele appears to be due to the *UGT1A* haplotype of variants located in the *UGT1A1* and *UGT1A3* genes. It remains to be determined whether these alleles or others that are in linkage disequilibrium with the *TA7* allele have enhanced glucuronidation activities toward estrogens.

Our results for the *UGT2B4(E458)* allele, although not statistically significant, suggest that it may be associated with a somewhat higher breast density. We also observed a nonsignificant inverse association between mammographic breast density and the *UGT2B7(Y268)* allele. This enzyme, expressed in breast tissue, has been shown to glucuronidate catechol estrogens, particularly 4-hydroxyestrone, which is a major metabolite of E_2 and has been shown to be carcinogenic in breast and uterine tissues (36). Thibaudeau et al. (37) evaluated the effects of the *UGT2B7(Y268)* allele on the formation of 4-hydroxyestrone and 4-hydroxyestradiol glucuronides in human embryonic kidney cells and showed that the *Y268* allele was associated with a significant 2-fold increase in clearance of these glucuronides compared with the wild-type. Because higher circulating E_1 and E_2 and free E_2 concentrations have been shown to be associated with higher percent mammographic density (38), we hypothesized that the *UGT2B7(Y268)* allele would be associated with lower percent density. Our results, although not statistically significant, as well as those from previous experimental studies (37, 39) support this hypothesis.

There are several strengths and limitations of our study. Premenopausal women tend to have high breast density, so study participants were sampled based on a Breast Imaging Reporting and Data System classification score, which allowed us to obtain a wide range of breast densities (10). However, because most women were White and of high socioeconomic status and all were members of a health plan, our findings may be generalizable only to similar populations of women. Another limitation is our small sample size, which restricted our ability to examine rare genotypes or interactions between genotypes. We may not have had adequate power to detect differences in mammographic density measures for most of the genotypes. Post hoc power calculations, based on the distribution of breast density measures observed and sample sizes obtained in this study, showed that we had <80% power to detect differences between genotypes for seven of the eight genes in our study. Given that many comparisons were made, it is possible that some of the statistically significant findings may have occurred by chance. Finally, there may have been bias due to nonparticipation, and although it is conceivable that an association might exist between mammographic breast density and willingness to participate, it is unlikely that the genetic polymorphisms would be differentially associated with those who participated and those who did not.

Measuring all the polymorphisms involved in steroid hormone metabolism was beyond the scope of this study. Few studies have evaluated relationships between the *UGT* and *SULT* polymorphisms and hormonal biomarkers in healthy, premenopausal women, and results from our study can be used as important preliminary data for determining approaches for future, larger-scale molecular epidemiologic studies that aim to capture all the relevant sex hormone metabolizing enzymes.

In summary, in this population of premenopausal women, mammographic breast density was significantly associated with polymorphisms in *SULT1A1*. Given that only one other study has examined the association between a *UGT* polymorphism and a biomarker of risk of hormone-dependent conditions in premenopausal women (33), larger studies examining the role of polymorphisms in steroid hormone pathway genes as predictive markers of mammographic breast density are needed. If the discovery of susceptibility genes is successful, the identification of high-risk women for prevention efforts by the use of multigenic models of breast cancer susceptibility may be possible (40).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Kelly Ehrlich, Kathy Plant, and the Group Health Research Institute for screening interviews, clinic visits, and study coordination and all of the study participants.

Grant Support

NIH grants R03CA121872 and R01CA97366 and the Fred Hutchinson Cancer Research Center interdisciplinary fellowship.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 9/2/09; revised 11/2/09; accepted 12/8/09; published online 2/8/10.

References

1. Cancer facts and figures—2009. Atlanta (GA): American Cancer Society; 2009.
2. Kelsey JL, Gammon MD, John EM. Reproductive factors and breast cancer. Epidemiol Rev 1993;15:36–47.
3. Eliassen AH, Missmer SA, Tworoger SS, et al. Endogenous steroid hormone concentrations and risk of breast cancer among premenopausal women. J Natl Cancer Inst 2006;98:1406–15.
4. Bergkvist L, Tabar L, Bergstrom R, Adami HO. Epidemiologic determinants of the mammographic parenchymal pattern. A population-based study within a mammographic screening program. Am J Epidemiol 1987;126:1075–81.
5. Boyd NF, Lockwood GA, Byng JW, Tritchler DL, Yaffe MJ. Mammographic densities and breast cancer risk. Cancer Epidemiol Biomarkers Prev 1998;7:1133–44.
6. de Waard F, Rombach JJ, Collette HJA, Slotboom B. Breast cancer risk associated with reproductive factors and breast parenchymal patterns. J Natl Cancer Inst 1984;72:1277–82.
7. Meech R, Mackenzie PI. Structure and function of uridine diphosphate glucuronosyltransferases. Clin Exp Pharmacol Physiol 1997; 24:907–15.
8. Falany CN. Enzymology of human cytosolic sulfotransferases. FASEB J 1997;11:206–16.
9. Atkinson C, Newton KM, Bowles EJ, Yong M, Lampe JW. Demographic, anthropometric, and lifestyle factors and dietary intakes in relation to daidzein-metabolizing phenotypes among premenopausal women in the United States. Am J Clin Nutr 2008;87: 679–87.
10. Atkinson C, Newton KM, Aiello Bowles EJ, et al. Daidzein-metabolizing phenotypes in relation to mammographic breast density among premenopausal women in the United States. Breast Cancer Res Treat 2009;116:587–94.
11. Adjei AA, Thomae BA, Prondzinski JL, Eckloff BW, Wieben ED, Weinshilboum RM. Human estrogen sulfotransferase (SULT1E1) pharmacogenomics: gene resequencing and functional genomics. Br J Pharmacol 2003;139:1373–82.
12. Lampe JW, Bigler J, Horner NK, Potter JD. UDP-glucuronosyltransferase (UGT1A1*28 and UGT1A6*2) polymorphisms in Caucasians and Asians: relationships to serum bilirubin concentrations. Pharmacogenetics 1999;9:341–9.
13. Thomas SS, Li SS, Lampe JW, Potter JD, Bigler J. Genetic variability, haplotypes, and htSNPs for exons 1 at the human UGT1A locus. Hum Mutat 2006;27:717.
14. Lampe JW, Bigler J, Bush AC, Potter JD. Prevalence of polymorphisms in the human UDP-glucuronosyltransferase 2B family: UGT2B4 (D458E), UGT2B7(H268Y), and UGT2B15(D85Y). Cancer Epidemiol Biomarkers Prev 2000;9:329–33.
15. Raftogianis R, Wood TC, Weinshilboum RM. Human phenol sulfotransferases SULT1A2 and SULT1A1: genetic polymorphisms, allozyme properties, and human liver genotype-phenotype correlations. Biochem Pharmacol 1999;58:605–16.
16. Gallagher CJ, Muscat JE, Hicks AN, et al. The UDP-glucuronosyltransferase 2B17 gene deletion polymorphism: sex-specific association with urinary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol glucuronidation phenotype and risk for lung cancer. Cancer Epidemiol Biomarkers Prev 2007;16:823–8.
17. Lin DY, Zeng D. Likelihood-based inference on haplotype effects in genetic association studies. J Am Stat Assoc 2006;101:89–104.
18. Sillanpää P, Kataja V, Eskelinen M, et al. Sulfotransferase 1A1 genotype as a potential modifier of breast cancer risk among premenopausal women. Pharmacogenet Genom 2005;15:749–752.
19. Carlini EJ, Raftogianis RB, Wood TC, et al. Sulfation pharmacogenetics: SULT1A1 and SULT1A2 allele frequencies in Caucasian, Chinese and African-American subjects. Pharmacogenetics 2001; 11:57–68.
20. Habel LA, Capra AM, Oestreicher N, et al. Mammographic density in a multiethnic cohort. Menopause 2007;14:891–9.
21. Nagar S, Walther S, Blanchard R. Sulfotransferase (SULT) 1A1 polymorphic variants *1, *2, and *3 are associated with altered enzymatic activity, cellular phenotype and protein degradation. Mol Pharmacol 2006;69:2084–92.
22. Bradlow HL, Telang NT, Sepkovic DW, Osborne MP. 2-Hydroxyestrone: the 'good' estrogen. J Endocrinol 1996;150:S259–65.
23. Fishman J, Martucci C. Biological properties of 16α-hydroxyestrone: implications in estrogen physiology and pathophysiology. J Clin Endocrinol Metab 1980;51:611–5.
24. Riza E, dos Santos Silva I, De Stavola B, et al. Urinary estrogen metabolites and mammographic parenchymal patterns in postmenopausal women. Cancer Epidemiol Biomarkers Prev 2001;10:627–34.
25. Muti P, Bradlow HL, Micheli A, et al. Estrogen metabolism and risk of breast cancer: a prospective study of the 2:16α-hydroxyestrone ratio in premenopausal and postmenopausal women. Epidemiology 2000; 11:635–40.
26. Hui Y, Yasuda S, Liu M-Y, We Y-Y, Liu M-C. On the sulfation and methylation of catecholestrogens in human mammary epithelial cells and breast cancer cells. Biol Pharm Bull 2008;31:769–73.
27. Spink BC, Katz BH, Hussain MM, et al. SULT1A1 catalyzes 2-methoxyestradiol sulfonation in MCF-7 breast cancer cells. Carcinogenesis 2000;21:1947–57.
28. Yang G, Gao YT, Cai QY, Shu XO, Cheng JR, Zheng W. Modifying effects of sulfotransferase 1A1 gene polymorphism on the association of breast cancer risk with body mass index or endogenous steroid hormones. Breast Cancer Res Treat 2005;94:63–70.
29. Bosma P, Chowdhury JR, Bakker C, et al. The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. N Engl J Med 1995;333:1171–5.
30. Monaghan G, Ryan M, Seddon R, Hume R, Burchell B. Genetic variation in bilirubin UDP-glucuronosyltransferase gene promoter and Gilbert's syndrome. Lancet 1996;347:578–81.
31. Beutler E, Gelbart T, Demina A. Racial variability in the UDP-glucuronosyltransferase 1 (UGT1A1) promoter: a balanced polymorphism for regulation of bilirubin metabolism? Proc Natl Acad Sci U S A 1998;95:8170–4.
32. Senafi SB, Clarke DJ, Burchell B. Investigation of the substrate specificity of a cloned expressed human bilirubin UDP-gluronosyltransferase: UDP-sugar specificity and involvement in steroid and xenobiotic glucuronidation. Biochem J 1994;303:233–40.
33. Haiman CA, Hankinson SE, De Vivo I, et al. Polymorphisms in steroid hormone pathway genes and mammographic density. Breast Cancer Res Treat 2003;77:27–36.
34. Peters WHM, te Morsche RHM, Roelofs HMJ. Combined

polymorphisms in UDP-glucuronosyltransferases 1A1 and 1A6: implications for patients with Gilbert's syndrome. J Hepatol 2003;38:3–8.
35. Lankisch TO, Behrens G, Ehmer U, et al. Gilbert's syndrome and hyperbilirubinemia in protease inhibitor therapy—an extended haplotype of genetic variants increases risk in indinavir treatment. J Hepatol 2009;50:1010–8.
36. Bélanger A, Pelletier G, Labrie F, Barbier O, Chouinard S. Inactivation of androgens by UDP-glucuronosyltransferase enzymes in humans. Trends Endocrinol Metab 2003;14:473–9.
37. Thibaudeau J, Lépine J, Tojcic J, et al. Characterization of common UGT1A8, UGT1A9, and UGT2B7 variants with different capacities to inactivate mutagenic 4-hydroxylated metabolites of estradiol and estrone. Cancer Res 2006;66:125–33.
38. Greendale GA, Palla S, Ursin G, et al. The association of endogenous sex steroids and sex steroid binding proteins with mammographic density: results from the Postmenopausal Estrogen/Progestin Interventions Mammographic Density Study. Am J Epidemiol 2005;162:826–34.
39. Gestl SA, Green MD, Shearer DA, Frauenhoffer E, Tephly TR, Weisz J. Expression of UGT2B7, a UDP-glucuronosyltransferase implicated in the metabolism of 4-hydroxyestrone and all-trans retinoic acid, in normal human breast parenchyma and in invasive and *in situ* breast cancers. Am J Pathol 2002;160:1467–79.
40. Feigelson HS, Henderson BE. Future possibilities in the prevention of breast cancer: role of genetic variation in breast cancer prevention. Breast Cancer Res 2000;2:277–82.