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Associations between polymorphisms in glucuronidation and sulfation enzymes and mammographic breast density in premenopausal women in the United States

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Abstract

Objective—Sex hormones are metabolized to less active compounds via (i) glucuronidation, catalyzed by UDP-glucuronosyltransferases (UGT) and (ii) 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 and seventy five women ages 40–45 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 to women with the *SULT1A1(R213/R213)* genotype after controlling for ethnicity (p-value = 0.001). Breast density was 5% lower among women carrying at least one copy of the *UGT1A1 (TA7)-UG1A3(R11)-UGT1A3(A47)* haplotype compared to the *UGT1A1(TA6)-UG1A3(W11)-UGT1A3(V47)* haplotype (p-value = 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.

Keywords

UDP-glucuronosyltransferases; sulfotransferases; hormones; mammographic breast density; premenopausal women

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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 (T) 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) (7) and sulfation catalyzed by sulfotransferases (SULT) (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 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 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), UGT2B15(Y85)*] would be associated with decreased breast density (i.e., 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), SULT1A1(H213)*] would be associated with increased breast density (i.e., via higher lifelong hormone exposure).

Materials and methods

Study Population

As described in detail elsewhere (9), women were recruited from within Group Health (GH), a large integrated health plan in Washington State. Premenopausal women ages 40 – 45 years who had undergone a screening mammogram in the previous 10 months, and who were not taking exogenous hormones, were identified from the GH Breast Cancer Screening Program and recruited based on the Breast Imaging Reporting and Data System (B-RADS®) 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 which 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 (FHCRC) and GH, and all study participants provided written informed consent.

Mammographic breast density data

Each participant's most recent routine GH X-ray screening mammogram prior to her study visit was digitized using a Lumysis 85 scanner (Sunnyvale, CA). 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 (Qiagen, Valencia, CA). The concentration and purity were determined by spectroscopy at 260nm and 280nm. 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 polymerase chain reaction (PCR)-based methods, including size dependent-separation, restriction fragment length polymorphism (RFLP), sequencing, and fluorescent allelic discrimination (TaqMan™). For *SULT1E1*, we selected three SNPs, ((*SULT1E1(I169A>G)*, *SULT1E1[I1(−73)G>C]*, and *SULT1E1[I5(−10) C>G]*), which were found by Adjei et al., that 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 the initial and duplicate assays. Genotype calling was done both by machine and 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 performed as previously described (12). The fragments were analyzed using an ABI PRISM 3100 Genetic Analyzer and Genotyper®2.5 software (Applied Biosystems, Foster City, CA, USA).

UGT1A3(W11R), UGT1A3(V47A)

Genotyping of the two-residue amino acid substitutions *W11R* (rs3821242) and *V47A* (rs6431625) of UGT1A3 involved two steps (13). First, PCR reactions were performed to amplify the region of interest. Second, the PCR amplicons were sequenced. The sequence data were analyzed using the Sequencher 4.1™ (Gene Codes Corp., Ann Arbor, MI) 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 RFLP 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 232bp and 32 bp and the expected fragment size for the *E458* allele was 264bp.

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

We genotyped the polymorphisms in *UGT2B7* (rs7439366), *UGT2B15* (rs1902023), *SULT1A1* (rs9282861) (15), and *SULT1E1* (rs3775768, rs4149530, and rs1220702) (11), and a deletion in *UGT2B17* (16) using TaqMan™. Data were analyzed with SDS software (Applied Biosystems, Foster City, CA) 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 had been previously validated. Validation of the *UGT2B7* and *UGT2B15* assays was performed using DNA samples (n = 20–100) from other studies that had previously been genotyped by RFLP 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 chi-square 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 the *UGT1A3(W11R)* and *UGT1A3 (V47A)* polymorphisms (D′=0.98), and between the *UGT1A1(TA7)* and *UGT1A3(W11R)* (D′= −0.96) and the *UGT1A1(TA7)* and *UGT1A3(V47A)* (D′ = 0.98) polymorphisms. Because the functional effect of *UGT1A1(TA7)* is decreased UGT1A1 gene expression and therefore decreased glucuronidation and *UGT1A3(11R)* and *UGT1A3(47A)* result in increased glucuronidation, we inferred haplotypes involving these three loci for our study population. For each gene (*UGT1A*, *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 *UGT1A1-1A3* 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, 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 births category (0, 1–2, 3+) to compute the mean percent density values for the *SULT1A1(R213/H/213)* 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 wildtype (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 (i.e., 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 (i.e., White vs. 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 LP, College Station, TX), 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-value of < 0.05 was considered statistically significant.

Results

The mean age of the study participants was 42.4 (SD 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, intrauterine devices containing progesterone), were non-smokers (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 non-significant chi-square tests at $p<0.05$), are presented in Table 3.

After adjusting for ethnicity, women with the *UGT1A3(W11/R11)* and *UGT1A3(R11/R11)* genotypes had lower mean percent mammographic breast density compared to women with the wildtype [*UGT1A3(W11/W11)*] [35.1% and 31.6%, respectively vs. 40.9% (p-trend = 0.04)] (Table 3). There was a non-statistically significant inverse association between the *UGT1A1(TA7)-1A3(R11)-1A3(A47)* haplotype and mammographic breast density compared to the more common *UGT1A1(TA6)-1A3(W11)-1A3(V47)* haplotype in this population [35.7% vs. 40.6%, respectively; p-value = 0.07 (Table 3)]. Mean mammographic breast densities for women with the *SULT1A1(R213/H213)* and *SULT1A1(H213/H213)* genotypes were lower compared to women homozygous for *SULT1A1 R213* (34.9% and 25.7%, vs. 41.8%, respectively; p-trend = 0.001; Table 3). We also observed non-significant inverse associations between both the *TA7* allele of UGT1A1 and the *Y268* allele of UGT2B7 and mammographic density, and a non-significant 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 to noncarriers [percent density (95% CI): carriers: 33.6% (29.3% – 37.8%), noncarriers: 40.4% (35.0% – 45.8%); p-value = 0.050; data not shown]. No statistically significant associations between percent mammographic breast density and either *UGT2B15 (D85Y)* and the *UGT2B17deletion* were observed.

Of the three *SULT1E1* SNPs 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* SNPs, 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 (i.e., 0, 1–2, 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: *R213/H213* = −8.0%, *H213/H213* = −18.9%; p trend = 0.049) compared to 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-value = 0.83).

Discussion

In this well-characterized population of healthy, premenopausal women, we assessed the associations 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 to the wildtype [*SULT1A1(R213/R213)*] (21). Our results could, however, reflect the role of catechol estrogens [i.e., 2-hydroxy (OH) estrone (E_1) and 16α-OH E_1]. 2-OH E_1 is conjugated to anticarcinogenic methoxylated metabolites [e.g., 2-methoxy (MeO)E_1 and 2-MeOE_2] and is hypothesized to protect against breast cancer (22). In contrast, 16α-OH E_1 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-MeOE_2 sulfation (27), it is possible that SULT1A1 could modify the effects of 2-MeOE_2. Women with low activity *SULT1A1(H213/H213)* genotype could have higher levels of the unconjugated form of 2-MeOE_2. Consequently, the potential protective effects of this metabolite may be prolonged in women with low sulfation capacity compared to 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 non-significant inverse trend with the *UGT2B15 Y85* 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 SNPs 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 glucuronidationof estradiol (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 (LD) 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 V47* 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 impact on mammographic density in combination with the *UGT1A3 A47* allele, and only a small impact when inherited with the *UGT1A1 TA7* allele. These findings suggest that at the *UGT1A* locus, the co-inheritance of *UGT1A3 R11* and *UGT1A3 V47*, 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 LD with the *TA7* allele have enhanced glucuronidation activities towards 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 non-significant 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-OH E_1 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-OH E_1 and 4-OH E_2 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 to the wildtype. Because higher circulating E_1, 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 were several strengths and limitations of our study. Premenopausal women tend to have high breast density, so study participants were sampled based on a B-RADS® 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 less than 80% power to detect differences between genotypes for 7 of the 8 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 non-participation, 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).

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Table 1

Primer and/or probe sequences for genotyping

Polymorphism	Sequence
UGT1A1	
PCR primers	
FP	6FAM – GTC ACG TGA CAC AGT CAA AC 3′
RP	5′ GTT TCT TTT TGC TCC TGC CAG AGG TT 3′
UGT1A3	
PCR primers	
FP	5′ AGT GAG CAC AGG GTC AGA CGT 3′
RP	5′ TCC AGG ATG GAT CAG TTC CA 3′
Sequencing primers	
FP1	5′ GCT CAG TGA CAA GGT AAT TA 3′
RP1	5′ GAA GGC TAT TAT GAC AAG GA 3′
FP2	5′ CAC ACT CAA CTG TAC TTT GAA 3′
RP2	5′ CTT TGC ATG AAT GTC ATG T 3′
UGT2B4	
PCR primers	
FP	5′ TTC ATC ATG ATC AAC CAG TGA 3′
RP	5′ CTT CCA GCC TCA GAC GTA AT 3′
UGT2B7	
PCR primers	
FP	5′ GGC TTA TTC GAA ACT CCT GGA A 3′
RP	5′ TGG AGT CCT CCA ACA AAA TCA A 3′
Probes	
C	6FAM – AGT TTC CAc ATC CAC-MGBNFQ
T	VIC – TTT CCA tAT CCA CTC TT-MGBNFQ
UGT2B15	
PCR primers	
FP	5′ GCC AGT AAA TCA TCT GCT ATT AAA TTA GAA 3′

Polymorphism	Sequence
RP	5′ GCA TCT TTA CAG AGC TTG TTA CTG TAG TCA TA 3′
Probes	
T	6FAM – TCA GAA GAG AAT CTT CCA AAT AAT TT- MGBNFQ
G	VIC TCA GAA GAG AAT CTT CCA AAT CAT TT- MGBNFQ
UGT2B17	
PCR primers	
Exon1 FP	5′ TGA AAA TGT TCG ATA GAT GGA CAT ATA GTA 3′
Exon1 RP	5′ GAC ATC AAA TTT TGA CTC TTG TAG TTT TC 3′
Deletion FP	5′ TTT AAT GTT TTC TGC CTT ATG CCA C 3′
Deletion RP	5′ AGC CTA TGC AAT TTT CAT TCA ACA TAG 3′
Probes	
Exon1	6FAM – TAC ATT TTG GTC ATA TTT TTC ACA ACT ACA AGA ATT GT-MGBNFQ
Deletion	JOE – ACT ACA CTG AGA TTT ACA AAA GAA TTC TGT CAG GAT ATA G-MGBNFQ
SULT1A1	
PCR primers	
FP	5′ AGT TGG CTC TGC AGG GTT TCT 3′
RP	5′ ACC ACG AAG TCC ACG GTC TC 3′
Probes	
R	VIC – TGG CAG GGA GCG C-MGBNFQ
H	6FAM – CTG GCA GGG AGT GC-MGBNFQ

Table 2

Characteristics of premenopausal women in the study population: Group Health, Seattle, WA 2004 – 2005 (N = 175*)

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:Hip ratio		
Mean (SD)	0.79	(0.06)
Median (Range)	0.78	(0.66, 1.00)
	n	**(%)**
Number of 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 years	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)

*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 prior to the 6-month period before the screening mammogram

Table 3

Adjusted[§] mean percent mammographic density of study population by genotype and haplotype: Group Health, Seattle, WA 2004 – 2005

Genotype	n (%)	Mean[§] (95% CI)	p-value	p trend
*UGT1A1*28*				
TA_6/TA_6	91 (52.0)	39.0 (34.0, 44.1)	Reference	
TA_6/TA_7	69 (39.4)	35.0 (30.0, 39.9)	0.26	0.12
TA_7/TA_7	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	
W11/R11	74 (42.3)	35.1 (30.3, 40.0)	0.14	0.04
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	
V47/A47	71 (40.6)	33.2 (28.4, 37.9)	0.05	0.11
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	
D458/E458	68 (38.9)	38.2 (33.1, 43.4)	0.37	0.25
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	
H268/Y268	86 (49.1)	35.8 (31.0, 40.7)	0.29	0.22
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	
D85/Y85	94 (53.7)	36.2 (31.6, 40.8)	0.41	0.50
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	
not deleted/deleted	75 (42.9)	34.3 (29.3, 39.3)	0.31	0.80
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	
R213/H213	64 (36.6)	34.9 (29.6, 40.2)	0.08	0.001
H213/H213	28 (16.0)	25.7 (18.4, 33.0)	0.001	
Haplotype	**Frequency**			
UGT1A1(TA_6/TA_7)- 1A3(W11R)-1A3(V47A)				
TA_6-W11-V47	0.59	40.6 (33.9, 47.4)	Reference	
TA_6-R11-V47	0.06	32.4 (15.6, 49.4)	0.12	
TA_6-R11-A47	0.06	38.4 (22.5, 54.7)	0.66	N/A

Genotype	n (%)	Mean[§] (95% CI)	p-value	p trend
TA_7-R11-A47	0.28	35.7 (23.8, 47.8)	0.07	
SULT1E1: rs3775768 (A/G), rs4149530 (G/C)				
A-G	0.57	37.6 (31.1, 44.2)	Reference	
A-C	0.05	38.5 (18.5, 59.0)	0.87	N/A
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

Number of live births	SULT1A1 genotype	n[*] (%)	Mean[§] (95%CI)	Absolute % diff	p-value	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	

[*] number does not add up to 175 because 19 participants were missing data on number of live births;

[§] adjusted for ethnicity using least squares regression;

[†] p-value (interaction) = 0.83