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UDP-glucuronosyltransferase and sulfotransferase polymorphisms, sex hormone concentrations, and tumor receptor status in breast cancer patients

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Abstract

Introduction UDP-glucuronosyltransferase (UGT) and sulfotransferase (SULT) enzymes are involved in removing sex hormones from circulation. Polymorphic variation in five *UGT* and *SULT* genes – *UGT1A1* ($(TA)_6/(TA)_7$), *UGT2B4* (Asp[458]Glu), *UGT2B7* (His[268]Tyr), *UGT2B15* (Asp[85]Tyr), and *SULT1A1* (Arg[213]His) – may be associated with circulating sex hormone concentrations, or the risk of an estrogen receptor-negative (ER⁻) or progesterone receptor-negative (PR⁻) tumor.

Methods Logistic regression analysis was used to estimate the odds ratios of an ER⁻ or PR⁻ tumor associated with polymorphisms in the genes listed above for 163 breast cancer patients from a population-based cohort study of women in western Washington. Adjusted geometric mean estradiol, estrone, and testosterone concentrations were calculated within each *UGT* and *SULT* genotype for a subpopulation of postmenopausal breast cancer patients not on hormone therapy 2–3 years after diagnosis (*n* = 89).

Results The variant allele of *UGT1A1* was associated with reduced risk of an ER⁻ tumor (*P* for trend = 0.03), and variants of *UGT2B15* and *SULT1A1* were associated with non-statistically significant risk reductions. There was some indication that plasma estradiol and testosterone concentrations varied by *UGT2B15* and *SULT1A1* genotypes; women with the *UGT2B15* Asp/Tyr and Tyr/Tyr genotypes had higher concentrations of estradiol than women with the Asp/Asp genotype (*P* = 0.004). Compared with women with the *SULT1A1* Arg/Arg and Arg/His genotypes, women with the His/His genotype had elevated concentrations of testosterone (*P* = 0.003).

Conclusions The risk of ER⁻ breast cancer tumors may vary by *UGT* or *SULT* genotype. Further, plasma estradiol and testosterone concentrations in breast cancer patients may differ depending on some *UGT* and *SULT* genotypes.

Keywords: breast cancer, estrogen, glucuronosyltransferase, polymorphism, sulfotransferase, testosterone

Introduction

Exposure to increased concentrations of both estrogens and androgens has been implicated in the development of breast cancer [1-3]. There are varied mechanisms by which sex hormones might act to propagate malignancy; at the same time, there are only a few pathways by which these hormones are inactivated and removed from circulation.

Glucuronidation and sulfonation are two of the pathways through which sex hormones can be metabolized to inactive compounds [4-6].

Glucuronidation is catalyzed by UDP-glucuronosyltransferase (UGT) enzymes and involves the covalent addition of glucuronic acid, resulting in more hydrophilic compounds

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CI = confidence interval; CV = coefficient of variation; ER = estrogen receptor; HEAL = Health, Eating, Activity, and Lifestyle; OR = odds ratio; PR = progesterone receptor; SEER = Surveillance, Epidemiology, and End Results; SULT = sulfotransferase; UGT = UDP-glucuronosyltransferase.

that are excreted from the body via urine or bile [6,7]. UGT1A1, UGT2B4, UGT2B7, and UGT2B15 are among the UGT enzymes that metabolize steroid hormones [4].

The UGT1A1 protein glucuronidates estriol, 17β-estradiol, ethinylestradiol, and catechol estrogens [8-11]. Variation in glucuronidation between individuals is due primarily to a variable number of TA repeats in the $A(TA)_nTAA$ ($n = 5–8$) promoter sequence in the TATA-box region [12,13]. Among the variant alleles, $(TA)_7$ (the most common variant) and $(TA)_8$ are associated with lower transcriptional activity *in vitro* than the $(TA)_6$ wild-type allele [12-14]. An increasing number of TA repeats has been associated with increased breast cancer risk in premenopausal African-American women, but not postmenopausal African-American or premenopausal or postmenopausal Caucasian women [14,15].

The UGT2B4 enzyme glucuronidates catechol estrogens, estriol, and bile acids [16-18]. A polymorphism in the *UGT2B4* gene (Asp[458]Glu) has been reported [16]. The two alleles have similar tissue distributions and currently there is little biochemical evidence to suggest different substrate specificities between the two isoforms [16,19]. However, it has not yet been determined whether this polymorphism affects UGT2B4 protein function through other mechanisms. The UGT2B7 enzyme glucuronidates a wide variety of steroids, including catechol estrogens, estriol, and hydroxylated androgens [10,17,18,20-25]. A polymorphism in the *UGT2B7* gene results in an amino acid change (His[268]Tyr) [22], possibly near the proposed substrate-binding site of the protein [22,26]. The two alleles seem to have similar enzymatic activities for most substrates [10,24,27], with the possible exception of androsterone, estriol, and estradiol [21-23,25]. The UGT2B15 enzyme conjugates catechol estrogens and the C_{19} sex steroids testosterone, dihydrotestosterone, and androstane-3α, 17β-diol [18,28-30]. A polymorphism in the *UGT2B15* gene (Asp[85]Tyr) [28] results in an allele with a V_{max} for androstane-3α, 17β-diol and dihydrotestosterone *in vitro* that is double that of the aspartic acid allele [28].

Sulfotransferases (SULTs) comprise another group of enzymes involved in the removal of circulating bioactive sex hormones. The SULT1A1 enzyme can catalyze the sulfonation of estrogens to form inactive estrogen sulfates [6,31,32]. It has been proposed that estrone sulfate serves as an inactive reservoir in the blood from which estrone and estradiol can be regenerated [6,33]. A polymorphism in the *SULT1A1* gene (Arg[213]His) has been identified [34]. Individuals homozygous for the histidine allele have lower platelet SULT activity *in vitro* than wild-type and heterozygous individuals [34,35]. Further, the *SULT1A1* variant allele is associated with an elevated breast cancer risk among postmenopausal women [36].

Because the UGT and SULT enzymes are important in the inactivation and removal of bioactive sex hormones from target tissues, investigating the association between polymorphisms in these genes and circulating sex hormone concentrations might help to elucidate the potential functional relevance of these polymorphisms *in vivo*. These observations might support experimental results suggesting a functional role for polymorphisms in *UGT1A1*, *UGT2B15*, and *SULT1A1*, or might generate new hypotheses about the potential effect of sequence variations in *UGT2B4* and *UGT2B7*, for which there is relatively little biochemical evidence suggesting a functional effect *in vitro*. With this purpose we investigated the association between these polymorphisms and circulating sex hormone concentrations in postmenopausal breast cancer patients not taking hormones 2–3 years after diagnosis. In view of increasing evidence that hormones are important in the development of hormone-dependent and hormone-independent breast cancer tumors [37-41], we also examined the association between polymorphisms in the *UGT1A1*, *UGT2B4*, *UGT2B7*, *UGT2B15*, and *SULT1A1* genes and breast tumor estrogen receptor (ER) and progesterone receptor (PR) status in newly diagnosed breast cancer patients.

Materials and methods
Study population
The Health, Eating, Activity, and Lifestyle (HEAL) study is a population-based, multicenter prospective cohort study in which women with breast cancer are followed to determine whether breast cancer prognosis is associated with diet, physical activity, sex hormones, weight, or other exposures [42]. Participants in the HEAL study were identified through Surveillance, Epidemiology, and End Results (SEER) registries in western Washington state, in New Mexico, and in Los Angeles County, California. The analyses presented here are limited to breast cancer cases in Washington state who were identified through the western Washington SEER cancer registry between 1996 and 1999. To be eligible, women had to be newly diagnosed with stage 0, I, II, or IIIa breast cancer, living in King, Pierce, or Snohomish counties, and to have clinic measurements and a blood draw within 4 to 12 months of diagnosis. Because the majority of women were either Caucasian or Asian, the analyses were restricted to these two races. Of 202 patients interviewed in western Washington, 184 women were Caucasian or Asian and had a blood draw at baseline. Of these women, the ER and PR status of the breast tumor was determined for 163 women. Women for whom ER and PR status were determined did not significantly differ from the 21 women with unknown receptor status with respect to characteristics presented in Table 1, with the exception that the latter women were more likely to have *in situ* breast cancer (31% versus 52%). A participant's menopausal status was determined by using self-

Table 1

Selected characteristics of the Caucasian and Asian women enrolled in the Health, Eating, Activity, and Lifestyle study and asubpopulation used for investigating associations with circulating sex hormones at the 24-month follow-up

Characteristic	Baseline ($n = 163$) n (%)	Subpopulation at 24-month follow-up[a] ($n = 89$) n (%)
Age at enrollment, years	52.4 (6.5)[b]	57.3 (5.7)[b]
40–49	59 (36)	10 (11)
50–59	75 (46)	43 (48)
60–69	29 (18)	36 (40)
Race		
Caucasian	150 (92)	82 (92)
Asian	13 (8)	7 (8)
Number of pregnancies		
lasting at least 6 months		n/a
0	31 (19)	
1	27 (17)	
2	59 (36)	
3+	45 (28)	
Weight change since age 18 (kg)	+15.8 (14.4)[b]	n/a
Body fat (%)	n/a	36.8 (5.5)[b]
Tumor receptor status		
ER$^+$ tumor	124 (76)	n/a
PR$^+$ tumor	128 (79)	n/a
ER$^+$/PR$^+$ tumor	107 (66)	n/a
ER$^+$/PR tumor	17 (10)	
ER /PR$^+$ tumor	21 (13)	
ER /PR tumor	18 (11)	
Stage of disease		
0, *in situ*	51 (31)	n/a
I	83 (51)	
II	29 (18)	
Subject genotypes		
UGT1A1 genotypes $(TA)_6/(TA)_7$		
$(TA)_6/(TA)_6$	80 (49)	49 (55)
$(TA)_6/(TA)_7$	72 (44)	36 (40)
$(TA)_7/(TA)_7$	11 (7)	4 (5)
$(TA)_7$ allele frequency	0.29	0.25
UGT2B4 genotypes (Asp^{458}Glu)		
Asp/Asp	104 (64)	53 (60)
Asp/Glu	46 (28)	29 (33)
Glu/Glu	13 (8)	7 (8)
Glu allele frequency	0.22	0.24

Table 1 *(Continued)*

Selected characteristics of the Caucasian and Asian women enrolled in the Health, Eating, Activity, and Lifestyle study and asubpopulation used for investigating associations with circulating sex hormones at the 24-month follow-up

UGT2B7 genotypes (His[268]Tyr)		
His/His	41 (25)	24 (27)
His/Tyr	73 (45)	48 (54)
Tyr/Tyr	49 (30)	17 (19)
Tyr allele frequency	0.52	0.46
UGT2B15 genotypes (Asp[85]Tyr)		
Asp/Asp	32 (20)	19 (21)
Asp/Tyr	83 (51)	47 (53)
Tyr/Tyr	48 (29)	23 (26)
Tyr allele frequency	0.55	0.52
SULT1A1 genotypes (Arg[213]His)		
Arg/Arg	71 (43)	39 (44)
Arg/His	68 (42)	38 (43)
His/His	24 (15)	12 (13)
His allele frequency	0.36	0.35

ER, estrogen receptor; n/a, not applicable; PR, progesterone receptor. [a]Restricted to postmenopausal women not taking estrogen or progesterone therapy at the time of blood draw for investigation of associations between genotype and sex hormone concentrations. [b]Mean (SD).

reported information on estrogen and tamoxifen use, prior ovarian surgery or hysterectomy, menstrual history, and measured values of estrone, estradiol, and follicle-stimulating hormone. At baseline, 61 women were classified as premenopausal, 98 as postmenopausal and 4 were unable to be classified. Data from a follow-up interview and blood draw that occurred 24 months after initial enrollment were used for the analysis of serum hormone concentrations. At the 24-month follow-up, 35 women were classified as premenopausal, 114 as postmenopausal, and 16 were unable to be classified. Because we were unable to collect blood samples from premenopausal women at the same point in the menstrual cycle, hormone analyses were limited to postmenopausal Caucasian and Asian women who had complete data for estrone, estradiol, height and weight, and who were not taking estrogen or progesterone replacement hormones at the time of the blood draw (*n* = 89). There was no correlation between hormone concentrations and time from breast cancer diagnosis to blood draw at the 24-month follow-up.

Written informed consent was obtained from each participant. The protocol was approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center.

Data collection
Data from two time points were used in this study. The first time point was the time of study enrollment, 4–12 months

after breast cancer diagnosis. The second time point was about 24 months after study enrollment. Patients were mailed study questionnaires to complete and bring with them to their clinic evaluations at the Fred Hutchinson Cancer Research Center Prevention Studies Clinic. Information was collected on the following: dietary intake; health habits; reproductive and menstrual history; history of use of oral contraceptives and hormone replacement therapy; medical history, including history of endocrine problems and other medical problems; history of benign breast disease; family history of breast cancer, other cancers, and diabetes mellitus; history of tobacco, caffeine, and alcohol use; lifetime weight patterns; detailed current and pre-diagnostic physical activity habits; mammographic screening; and selected demographic data. Standard height and weight measurements were obtained by trained staff during home or clinic visits. Percentage body fat was measured by bioelectric impedance (RJL Multifrequency Bioelectric Impedance Analyzer, Clinton Township, MI). Bioelectrical impedance measurements were made with standard electrode placements after a minimum 4-hour fast.

Hormone assays
A 30 ml fasting blood draw was collected at the 24-month follow-up visit to determine circulating concentrations of sex hormones. Blood was processed within 1 hour of collection; serum, plasma, and buffy coat aliquots were stored at -70 to -80°C. Dates of sample collection and processing,

time of day of blood collection, and time since last meal were recorded.

Estrone and estradiol assays were performed at Quest Diagnostics Nichols Institute (San Juan Capistrano, CA) between July and August 2001. The testosterone assay was performed in Dr Frank Stanczyk's laboratory at the University of Southern California between April and June 2002. Samples were assigned randomly to assay batches and ordered randomly within each batch. Laboratory personnel performing the assays were blinded to subject identity.

Estrone and estradiol assay methods consisted of organic solvent extraction, followed by Celite column partition chromatography before quantification by radioimmunoassay. The reported sensitivities of these assays are less than 10 pg/ml and less than 2 pg/ml, respectively. Testosterone was also measured by extraction, Celite column chromatography, and radioimmunoassay; this method has a sensitivity of 1 ng/dl. Twenty replicated samples and eight pooled quality-control samples (two samples per batch) were included in the estrone and estradiol blood assays. For estrone and estradiol, the intra-assay coefficients of variation (CVs) were 13.3% and 28.8%, respectively, and the total CV results were 13.3% and 29.1%, respectively. For testosterone, 20 replicated samples and 14 pooled quality-control samples (two samples per batch) were included in the blood assays. The intra-assay CV and total CV were both 9.6%. Four subjects had estrone measurements below the 10 pg/ml limit of detection. To calculate a representative estrone value for measurements below this detection limit, we fitted a truncated log-normal distribution to the observed estrone data. The truncation was considered at the lower end of the distribution and the cumulative proportion at the truncation point (namely, estrone = 10 pg/ml) was used as the likelihood contribution from each of the subjects with estrone below the detection limit. The maximum likelihood estimate of the mean of the lognormal distribution was 3.079 and that of the standard deviation was 0.453. Using these maximum likelihood estimates in the lognormal distribution, we calculated the mean estrone value less than 10 pg/ml to be 8.702 pg/ml. The four estrone measurements below the limit of detection were assigned this value.

Hormone receptor characterization

Paraffin-embedded breast tumor tissue samples from 163 women were tested for this study. ER and PR proteins were assessed by immunohistochemistry in a single laboratory without knowledge of other laboratory results, patient characteristics, or outcome. Tissue blocks were selected by reviewing all histologic slides for each case. Blocks for testing were selected for the presence of representative tumor and, when available, the presence of adjacent benign epi-

thelium (used as an internal positive control). Immunohistochemistry was performed with modified standard immunohistochemical techniques. In brief, 5 μm sections of tumor were cut onto glass slides and blocked for endogenous peroxidase. Slides were treated with microwaves in the presence of citrate buffer [43-45]. After washes, primary antibodies (monoclonal anti-PR clone 1A6 [NovoCastra Lab] [46,47] and monoclonal anti-ER clone ER1D5 [AMAC, Inc.]) [48-50] were applied to the sections and incubated for 1 hour. Slides were washed and appropriate biotinylated secondary antibody, diluted in accordance with the manufacturer's instructions (Vector Laboratories, Burlingame, CA), was applied for 30 minutes. Slides were incubated for 30 minutes with avidin–biotin complex, followed by diaminobenzidine with 8% $NiCl_2$ for 10 minutes; nuclei were counterstained with methyl green [51,52]. Tumor cells were scored positive if nuclear immunostaining was present and more than 5% of tumor cells had positive staining.

Genotyping

A 50 ml fasting blood draw was collected at the time of the baseline interview. Blood was processed into serum, plasma, and buffy coat fractions and aliquots were stored at -70 to -80°C. DNA for genotyping was extracted from the buffy coat fraction at the Core Specimen Processing Laboratory (Fred Hutchinson Cancer Research Center) with standard techniques.

SULT1A1 genotyping of the Arg[213]His polymorphism was performed with a restriction-fragment-length polymorphism assay. A *SULT1A1*-specific fragment containing the polymorphism was amplified in a 20 μl reaction containing 1 × GeneAmp buffer II (Applied Biosystems, Foster City, CA), 3 mM $MgCl_2$, 200 μM deoxynucleotide triphosphates, each primer at 200 nM (forward primer 5'-AGTTGGCTCT-GCAGGGTTTCT-3', reverse primer 5'-ACCACGAAGTC-CACGGTCTC-3'), 100 ng of genomic DNA, and 0.5 U of AmpliTaq DNA polymerase (Applied Biosystems). Cycling was as follows: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 59°C for 45 seconds, and 72°C for 45 seconds, and final extension at 72°C for 5 minutes. The amplified fragment was digested with *Hha*I and separated on a 2% NuSieve agarose gel (Cambrex, Rockland, ME). The fragment sizes were 160 and 40 base pairs for the wild-type allele, and 200 base pairs for the variant allele. For quality control purposes, genotyping was repeated for 10% of the samples for each genotype. There were no discrepancies between the two results. *UGT2B4* Asp[458]Glu, *UGT2B7* His[268]Tyr, *UGT2B15* Asp[85]Tyr, and *UGT1A1* [TA]$_n$ genotyping was performed as described previously [53,54].

Statistical methods

We addressed two separate research questions, using two different statistical models. We first examined whether genotype was related to the risk of developing an ER-negative (ER⁻) or PR-negative (PR⁻) breast cancer tumor. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated by using unconditional logistic regression analysis to evaluate the association between genotype and tumor receptor status. We adjusted our logistic regression analyses for variables for which we had *a priori* knowledge of potential contribution to breast cancer risk or to the development of hormone-dependent or hormone-independent breast tumors. Because of the small size of the study, the data were not sufficiently robust to employ specific methods to evaluate confounding. In addition to race (Caucasian/Asian), the following breast cancer risk factors were included as covariates in the analysis: age at the time of interview (continuous), age at menarche (continuous), number of ovaries remaining, number of pregnancies lasting at least 6 months (0, 1, 2, or 3 or more), menopausal status at time of baseline interview (premenopausal/postmenopausal), smoker at time of interview (yes/no), parity/age at first birth (nulliparous, age at first birth 26 or less, age at first birth more than 26), change in weight from age 18 to the age that the subject had most recently passed (35, 50, or 60 years old). Eight of the 163 women were excluded from the logistic regression analyses because of missing covariate data, leaving 155 women in the final model. Genotype indicator variables were created by using the wild-type genotype as the reference category in the regression models. Genotypes were also evaluated with dichotomous variables ('any wild-type allele' [wild-type and heterozygous individuals] versus homozygous variant individuals, and 'any variant allele' [heterozygous and homozygous variant individuals] versus wild-type individuals) when patterns suggested a dominant or recessive genotype effect, respectively. We evaluated menopausal status as a potential effect modifier but were unable to evaluate effect modification by race owing to small numbers. However, we modeled effects excluding Asian women to evaluate whether results were different when limited to Caucasian women. All logistic regression analyses were performed with SAS statistical software, version 8.2 (SAS Institute, Cary, NC).

Our second research question examined whether genotype was related to sex hormone concentrations. Linear regression analysis with robust variance estimates was used to evaluate the association between *UGT* and *SULT* genotypes and circulating sex hormone concentrations (estrone, estradiol, testosterone) in postmenopausal women not taking estrogen or progesterone replacement hormones at the time of the blood draw. Hormone concentrations were natural-logarithm transformed to approximate normal distributions. We adjusted the linear regression

analyses for variables for which we had *a priori* knowledge of potential contribution to sex hormone concentrations. Geometric mean hormone concentrations within each genotype were calculated after adjustment for age at the time of interview (continuous), percentage body fat (continuous), tamoxifen use at the time of blood draw (yes/no), alcohol use (yes/no), smoking (yes/no), number of ovaries remaining, race (Asian/Caucasian), and batch number (estrone and estradiol only). We evaluated tamoxifen use as a potential effect modifier. As with the logistic regression analyses, we evaluated whether restriction to Caucasian women altered the results. Of the 82 Caucasian and 7 Asian postmenopausal women with complete data for estrone, estradiol, height, and weight who were not taking estrogen or progesterone replacement hormones at the time of the blood draw, two were lacking body fat data and one was lacking data for plasma testosterone. These women were dropped from the multivariate linear regression analysis, resulting in 87 women with estradiol and estrone data, and 86 women with testosterone data. All linear regression analyses were performed with Stata statistical software, version 7 (Stata Corporation, College Station, TX).

Results

A total of 163 women (150 Caucasian and 13 Asian) from Washington state were enrolled in the HEAL study and had complete baseline interview and hormone receptor data. Most women were diagnosed with Stage I breast cancer (51%) (Table 1), were postmenopausal at the time of study enrollment (60%), and had no known family history of breast cancer (51%). Most of these women had an ER-positive (ER⁺) or PR-positive (PR⁺) breast tumor and 66% had a combined ER⁺/PR⁺ tumor.

In Caucasians ($n = 150$), the observed variant allele frequencies of each gene were as follows: *UGT1A1*, $(TA)_7 = 0.31$; *UGT2B4*, Glu⁴⁵⁸ = 0.24; *UGT2B7*, Tyr²⁶⁸ = 0.55; *UGT2B15*, Tyr⁸⁵ = 0.56; *SULT1A1*, His²¹³ = 0.38. These allele frequencies were similar to those in Caucasians in previous reports [13,34,53-56]. We did not calculate separate allele frequencies in Asians because of the small number of individuals ($n = 13$). In Caucasians, genotype frequencies for each gene did not deviate from Hardy–Weinberg equilibrium.

As shown in Table 2, the risk of an ER⁻ breast tumor seemed smaller for individuals with the *UGT1A1* variant allele (compared with $(TA)_6/(TA)_6$: OR $(TA)_6/(TA)_7 = 0.6$, 95% CI 0.2–1.3; OR $(TA)_7/(TA)_7 = 0.0$, 95% CI 0.0–0.5; *P* for trend = 0.03). Further, a reduced risk of an ER⁻ tumor might be associated with the *SULT1A1* variant allele (OR = 0.6, 95% CI 0.3–1.2) and the *UGT2B15* homozygous variant genotype (OR = 0.4, 95% CI 0.1–1.2). The effects were similar when the sample was limited to Caucasian

Table 2

UGT1A1, UGT2B4, UGT2B7, UBT2B15, and SULT1A1 genotypes and risk of ER- and PR- breast tumors in Caucasian and Asian female breast cancer patients, Washington state

Gene	ER+ n	ER- n	OR[a] ER-tumor	Combined[b]	PR+ n	PR- n	OR[a] PR-tumor	Combined[b]
UGT1A1								
$(TA)_6/(TA)_6$	53	21	1.0 (ref)	1.0 (ref)	57	17	1.0 (ref)	1.0 (ref)[e]
$(TA)_6/(TA)_7$	55	16	0.6 (0.2–1.3)		55	16	1.1 (0.5–2.6)	
$(TA)_7/(TA)_7$	10	0	0.0 (0.0–0.5)*	0.5 (0.2–1.1)[d]	9	1	0.3 (0.02–2.6)	0.2 (0.02–2.4)
UGT2B4								
Asp/Asp	78	21	1.0 (ref)	1.0 (ref)	81	18	1.0 (ref)	
Asp/Glu	30	13	1.5 (0.6–3.6)		30	13	2.2 (0.9–5.4)	
Glu/Glu	10	3	1.3 (0.3–5.9)	1.5 (0.6–3.3)[d]	10	3	0.9 (0.2–4.5)	
UGT2B7								
His/His	31	9	1.0 (ref)		33	7	1.0 (ref)	
His/Tyr	58	12	0.6 (0.2–1.7)		52	18	1.5 (0.5–4.5)	
Tyr/Tyr	29	16	1.8 (0.6–5.2)		36	9	1.4 (0.4–4.5)	
UGT2B15								
Asp/Asp	20	10	1.0 (ref)	1.0 (ref)	25	5	1.0 (ref)	
Asp/Tyr	61	19	0.5 (0.2–1.4)		59	21	1.3 (0.4–4.1)	
Tyr/Tyr	37	8	0.4 (0.1–1.2)	0.5 (0.2–1.2)[d]	37	8	0.6 (0.2–2.4)	
SULT1A1								
Arg/Arg	49	19	1.0 (ref)	1.0 (ref)	52	16	1.0 (ref)	1.0 (ref)
Arg/His	50	13	0.6 (0.2–1.3)		50	13	0.9 (0.4–2.2)	
His/His	19	5	0.6 (0.2–1.9)	0.6 (0.3–1.2)[d]	19	5	0.8 (0.2–2.6)	0.9 (0.4–2.0)[d]

ER, estrogen receptor; OR, odds ratio; PR, progesterone receptor. [a]Adjusted for age, menopausal status, age at menarche, number of pregnancies lasting at least 6 months (0, 1, 2, or 3 or more), parity/age at first birth (nulliparous, age at first birth 26 or less, age at first birth more than 26), weight change since age 18, race (Asian/Caucasian), smoking, and number of ovaries remaining. [b]Genotypes were also evaluated with the use of dichotomous variables ('any wild-type allele' [wild-type and heterozygous individuals] and 'any variant allele' [heterozygous and homozygous variant individuals]) when patterns suggested a dominant or recessive genotype effect. [c]P for trend = 0.03. [d]OR for combined group of heterozygous and homozygous variant individuals. [e]OR for combined group of wild type and heterozygous individuals.

women (data not shown). Trends for *UGT1A1*, *UGT2B7*, *UGT2B15*, and *SULT1A1* were generally similar among premenopausal and postmenopausal women (data not shown).

Among the subpopulation of women eligible for hormone analysis (n = 89; Table 1), most (62%) were taking tamoxifen, but very few were current smokers (n = 4). Among this subgroup, women with the *UGT1A1* $(TA)_7/(TA)_7$ genotype had 58% higher geometric mean estradiol concentrations (P = 0.01) than women with the $(TA)_6/(TA)_6$ wild-type genotype (Table 3) and 71% higher geometric mean estradiol concentrations (P = 0.002) than the combined group of women with either the $(TA)_6/(TA)_6$ or the $(TA)_6/(TA)_7$ genotype (9.8 pg/ml, 95% CI 8.7–11.1 versus 16.8 pg/ml, 95% CI 12.6–22.5). Women with the *UGT2B15* Asp/Tyr and Tyr/Tyr genotypes had 59% (P =

0.003) and 44% (P = 0.05) higher geometric mean estradiol concentrations than women with the Asp/Asp genotype, respectively (Table 3). When genotypes were combined, women with any Tyr[85] allele had 53% higher geometric mean estradiol concentrations than Asp/Asp wild-type women with no Tyr[85] allele (P = 0.004; 7.2 pg/ml, 95% CI 5.6–9.4 versus 11.1 pg/ml, 95% CI 9.8–12.5). Finally, women with the *SULT1A1* His/His genotype had 32% higher geometric mean testosterone concentrations than women with the wild-type Arg/Arg genotype (P = 0.03; Table 3), and 39% higher geometric mean testosterone concentrations than the combined group of women with the Arg/Arg or Arg/His genotype (P = 0.003; 26.4 ng/dl, 95% CI 24.3–28.7 versus 36.8 ng/dl, 95% CI 30.5–44.3). No statistically significant associations were observed between estradiol, estrone or testosterone concentrations and *UGT2B4* or *UGT2B7* genotypes. Results

Table 3

Geometric mean estradiol (pg/ml), estrone (pg/ml), and testosterone (ng/dl) concentrations by genotype among postmenopausal Caucasian and Asian women with breast cancer in Washington state

Genotype	Estradiol			Estrone			Testosterone		
	n	Geometric mean[a] (95% CI)	Percentage difference (P)	n	Geometric mean[a] (95% CI)	Percentage difference (P)	n	Geometric mean[a] (95% CI)	Percentage difference (P)
UGT1A1									
(TA)$_6$/(TA)$_6$ (wt)	47	10.5 (9.1–12.1)	(Reference)	47	23.4 (20.9–26.3)	(Reference)	46	27.8 (24.9–31.0)	(Reference)
(TA)$_6$/(TA)$_7$ (het)	36	9.0 (7.3–11.2)	-14.1 (0.26)	36	21.1 (17.8–24.9)	-10.1 (0.31)	36	27.2 (23.8–31.2)	-1.9 (0.83)
(TA)$_7$/(TA)$_7$ (var)	4	16.6 (12.3–22.4)	+58.0 (0.01)	4	33.8 (19.9–57.4)	+44.1 (0.18)	4	29.9 (23.4–38.1)	+7.5 (0.60)
UGT2B4									
Asp/Asp (wt)	51	9.5 (8.2–11.1)	(Reference)	51	22.3 (19.4–25.6)	(Reference)	50	27.3 (24.5–30.4)	(Reference)
Asp/Glu (het)	29	11.0 (8.9–13.5)	+15.3 (0.29)	29	23.4 (19.6–28.1)	+5.2 (0.67)	29	28.5 (24.6–33.0)	+4.3 (0.67)
Glu/Glu (var)	7	10.9 (7.3–16.2)	+14.4 (0.53)	7	24.3 (18.2–32.5)	+9.2 (0.59)	7	26.6 (19.9–35.4)	-2.8 (0.86)
UGT2B7									
His/His (wt)	24	9.8 (7.5–12.8)	(Reference)	24	21.5 (17.8–26.0)	(Reference)	24	26.9 (23.3–31.0)	(Reference)
His/Tyr (het)	48	11.1 (9.6–12.9)	+13.3 (0.42)	48	24.0 (21.2–27.2)	+11.5 (0.34)	48	29.1 (26.2–32.3)	+8.2 (0.37)
Tyr/Tyr (var)	15	7.8 (5.6–10.7)	-20.9 (0.31)	15	21.3 (16.1–28.3)	-0.9 (0.96)	14	24.4 (19.4–30.7)	-9.2 (0.51)
UGT2B15									
Asp/Asp (wt)	19	7.2 (5.6–9.3)	(Reference)	19	20.7 (16.1–26.5)	(Reference)	18	26.8 (21.3–33.6)	(Reference)
Asp/Tyr (het)	45	11.5 (9.9–13.3)	+58.7 (0.003)	45	23.7 (20.9–26.8)	+14.6 (0.34)	45	29.0 (26.1–32.2)	+8.3 (0.55)
Tyr/Tyr (var)	23	10.4 (8.2–13.1)	+43.7 (0.05)	23	23.1 (18.3–29.0)	+11.6 (0.53)	23	25.8 (22.0–30.3)	-3.6 (0.78)
SULT1A1									
Arg/Arg (wt)	37	9.9 (7.8–12.6)	(Reference)	37	24.1 (20.0–29.2)	(Reference)	37	27.7 (23.9–32.0)	(Reference)
Arg/His (het)	38	9.8 (8.3–11.6)	-1.5 (0.93)	38	21.3 (18.6–24.3)	-12.0 (0.30)	37	25.2 (22.7–27.9)	-9.0 (0.32)
His/His (var)	12	11.6 (8.8–15.4)	+17.2 (0.42)	12	24.0 (19.3–29.9)	-0.6 (0.97)	12	36.5 (30.3–44.0)	+32.0 (0.03)

CI, confidence interval; het, heterozygous; var, homozygous variant; wt, wild-type. [a]Geometric mean estimates are for an individual with mean values of all covariates; adjusted for age, use of tamoxifen at the time of blood draw, smoking, alcohol use, percentage body fat, batch (estrone and estradiol only), number of ovaries remaining, and race (Asian/Caucasian).

were similar when the analyses were restricted to Caucasians (data not shown). We did not observe that the relationship between any of the polymorphisms and circulating sex hormone concentrations differed between tamoxifen users and non-users (data not shown), but the small number of individuals within each subgroup limited our ability to observe a difference.

Discussion

This study of female breast cancer patients had two aims: first, to evaluate the risk of ER- or PR- tumors associated with polymorphisms in specific *UGT* and *SULT* genes, and second, to investigate whether plasma sex hormone concentrations varied within genotypes of these same genes. To our knowledge this is the first study to investigate the

association between polymorphisms in the *UGT2B4*, *UGT2B7*, and *UGT2B15* genes and risk of an ER- or PR- breast tumor.

There is increasing evidence that hormones are important in the development of hormone-dependent and hormone-independent breast cancer tumors [37-41]. Women with ER- tumors have a worse prognosis and fewer treatment modalities are available. We observed a reduced risk of an ER- tumor in patients with the *UGT1A1* (TA)$_7$/(TA)$_7$ genotype, and indications towards risk reduction with variants of *UGT2B15* and *SULT1A1*. The association between breast tumor ER status and the number of TA repeats in the *UGT1A1* promoter region has previously been examined in a case-control study of 200 African-American women with breast cancer [14]. In that study, premenopausal women with (TA)$_7$ and (TA)$_8$ 'low-activity' alleles seemed to be at higher risk for an ER- tumor than women with (TA)$_5$ and (TA)$_6$ 'high-activity' alleles (OR = 2.1, 95% CI 1.0–4.2) [14]. This elevated risk did not extend to postmenopausal women (OR = 0.8, 95% CI 0.3–1.9), which is consistent with our results among a predominantly postmenopausal population. Our finding of a somewhat reduced risk of an ER- or PR- tumor in women with the *SULT1A1* Arg/His and His/His genotypes is consistent with the results of a study of 337 breast cancer patients by Nowell and colleagues [57]. Given that the hormonal milieu seems important for the development of both hormone-dependent and hormone-independent mammary tumors, our study, in conjunction with others, provides evidence that genotypes relevant to the metabolism and excretion of sex hormones might affect that milieu.

In a substudy of postmenopausal women at least 2 years after diagnosis, we observed that plasma estradiol concentrations varied by *UGT1A1* and *UGT2B15* genotypes. The *UGT1A1* gene is involved in the glucuronidation of several sex hormones, including 17β-estradiol [9,11]. Our finding that breast cancer patients homozygous for the *UGT1A1* variant (TA)$_7$ allele seemed to have increased concentrations of estradiol is consistent with the observation that the variant allele has lower transcriptional activity *in vitro* than the wild-type (TA)$_6$ allele [12-14]. However, a study of 274 healthy postmenopausal women in the Nurses' Health Study found that neither estrone nor estradiol concentration varied depending on *UGT1A1* genotype [15]. Our study was restricted to women with breast cancer, the majority of whom were on tamoxifen therapy, which alters estrogen levels, perhaps independently of these genetic factors. Our findings should therefore be confirmed in additional populations of breast cancer patients and healthy controls, to clarify the relationship between the *UGT1A1* polymorphism and estradiol concentrations in both healthy women and those with breast cancer.

Our finding that concentrations of estradiol were higher in women with the *UGT2B15* Tyr[85] allele is surprising, given that the UGT2B15 protein is not known to glucuronidate estradiol [18,29]. Further, we did not observe that circulating testosterone concentrations varied greatly by *UGT2B15* genotype, despite the fact that UGT2B15 is known to glucuronidate testosterone [18,29]. However, this might be explained by the much higher glucuronidation activity of UGT2B17 for testosterone than that of UGT2B15 [18,58]. Also surprising was the finding that the *SULT1A1* His/His genotype was associated with elevated testosterone concentrations, given that SULT1A1 does not seem to conjugate testosterone [32]. It is possible that the observed associations between sex hormone concentrations and polymorphisms in *UGT2B15* and *SULT1A1* in breast cancer patients are valid. However, they should be viewed as possibly attributable to chance, because there are conflicting biochemical data reported in the literature.

With regard to the *UGT2B4* and *UGT2B7* polymorphisms, there was relatively little biochemical evidence to support an *a priori* hypothesis about an association between these polymorphisms and circulating sex hormone concentrations or tumor receptor status. We therefore considered these analyses to be 'hypothesis generating', in which any observed association might elucidate the potential functional relevance of these polymorphisms *in vivo*. We did not find evidence that these polymorphisms were associated with sex hormone concentrations or the risk of an ER- or PR- breast cancer tumor. Our findings therefore support *in vitro* data in which these sequence variations did not seem to alter enzyme function [10,16,24,27].

Because genetic factors that might influence circulating sex hormone concentrations in breast cancer patients could influence response to treatment, it is important to understand these associations in breast cancer patients. The results of this study might serve as a starting point for the formation and testing of additional hypotheses regarding the potential association between polymorphisms in the *UGT2B4*, *UGT2B7*, and *UGT2B15* genes and serum hormone concentrations or hormone receptor status. One strength of the study is the investigation of several genetic polymorphisms in genes relevant to sex hormone excretion or regulation and the measurement of three sex hormones.

However, this study has several limitations. The small size limited the statistical power in many of the analyses and prohibited the evaluation of combined genotype effects. Additionally, the coefficients of variation for the estrone and estradiol assays were somewhat high, possibly preventing us from detecting an association. Another limitation is that we examined polymorphisms in only five genes whose protein products participate in sex hormone regulation; additional proteins involved in regulatory mechanisms that

contribute to sex hormone concentrations *in vivo* could not be considered as part of the study. As with any genetic association study, the results must be interpreted in the light of the possibility that observed associations might be due to linkage disequilibrium between the examined polymorphism and a functional polymorphism that is the true cause of the observed difference. Finally, because this study involved mostly overweight women with breast cancer, most of whom were taking tamoxifen at the time of the study, the observed associations between *UGT* and *SULT* genotypes and circulating hormone levels might not be generalizable to healthy women.

Conclusions
The results of this study of female breast cancer patients indicate that the risk of ER⁻ tumors varies by *UGT1A1*, and possibly *UGT2B15* and *SULT1A1*, genotype. Further, there was some indication that plasma estradiol and testosterone concentrations varied by *UGT1A1*, *UGT2B15*, or *SULT1A1* genotype. Owing to the preliminary nature of these findings, they should be validated in a larger study population.

Competing interests
None declared.

Acknowledgements

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