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Associations between the *CYP17, CYPIB1, COMT* and *SHBG* polymorphisms and serum sex hormones in postmenopausal breast cancer survivors

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Abstract

Several polymorphisms have been identified in genes that code for enzymes involved with estrogen biosynthesis and metabolism. Little is known about the functional relevance of these polymorphisms on sex hormones *in vivo*. We examined the association between *CYP17, CYP1B1, COMT* or *SHBG* genotypes and serum concentrations of estrone, estradiol, free estradiol, sex hormone binding globulin (SHBG), testosterone, free testosterone and dehyroepiandrosterone in 366 post-menopausal breast cancer survivors in New Mexico, California and Washington. Hormone levels were determined by high performance liquid chromatography and radioimmunoassay in blood drawn approximately 2 years post-diagnosis. We used generalized linear regression to calculate mean hormone levels by genotype, adjusting for age, race/ethnicity, stage, study site, tamoxifen use, number of remaining ovaries, hormone therapy use, marital status and BMI. No associations were observed between any of the genotypes and sex hormones when analyzing the main effects. In subgroup analyses, androgen levels of Hispanic women with the variant (A2) *CYP17* genotype were 46–87% higher than those of women with the wildtype; androgen levels were 13–20% lower in non-Hispanic whites with the variant genotype; no difference by genotype was observed for African-American women. Current tamoxifen users with the variant asn^{327} *SHBG* genotype had 81% higher serum SHBG and 39% lower free testosterone concentrations than women with the wildtype genotype. Non-tamoxifen users with the variant

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SHBG allele had elevated free estradiol levels. These results provide little evidence that the *CYP17*, *CYP1B1*, and *COMT* polymorphisms are associated with different sex hormone levels in post-menopausal breast cancer survivors.

Keywords

breast cancer; COMT; CYP17; CYP1B1; estrogen; SHBG; testosterone

Introduction

Cumulative exposure to estrogen is thought to be an important risk factor in the development of breast cancer in women. Estrogenic compounds can lead to increased cell proliferation and possibly act as DNA damaging agents [1–4]. Elevated serum estrogen concentration is a risk predictor for postmenopausal breast cancer [5]. In addition to influencing disease risk, estrogen-related factors may also impact progression as evidenced by the anti-estrogenic role of the selective estrogen-receptor modulator, tamoxifen, in improving disease prognosis among patients with estrogen receptor (ER)-positive tumors [6]. Many studies have reported poorer survival for factors related to high estrogen levels such as obesity [7].

Recent evidence suggests that oxidative metabolites of estrogen, particularly catechol estrogens [3], can form reactive quinones. These compounds may cause DNA adducts and are capable of redox cycling, which generates other reactive oxidative compounds [4]. Thus, understanding the genetic and environmental factors influencing estrogen biosynthesis and subsequent metabolism is important in understanding breast cancer etiology.

Several single nucleotide polymorphisms (SNPs) have been identified in genes that code for enzymes involved with estrogen biosynthesis (*CYP17*), metabolism (*CYP1B1* and *COMT*), and bioavailability (*SHBG*). The *CYP17* gene codes for the cytochrome p450C17α enzyme, a 17α-hydroxylase that catalyzes the conversion of pregnenolone and progesterone to 17-OH-Pregnenolone and 17-OH-progesterone, respectively [8]. It also has a 17,20-lyase activity that converts the hydroxylase products to dehydroepiandrosterone (DHEAS) and androstenedione, respectively, which can be further converted to estrogens and testosterone [8]. A variant identified in the 5’ untranslated region is predicted to enhance enzyme activity [9].

CYP1B1 and *COMT* are involved in metabolizing estrogenic compounds. *CYP1B1* is found at high levels in breast tissue and catalyzes the C4 hydroxylation of estradiol [10]. The resultant 4-OH catechol estrogen is potentially carcinogenic [11]. The m1 allele, a leucine to valine substitution at codon 432 ($Leu^{432}Val$), is common in Caucasians and African-Americans, and may have a higher catalytic activity than the wildtype allele [12,13]. The *COMT* gene encodes catechol-O-methyl-transferase, which converts catechol estrogens into inactive metabolites [11]. Approximately 25% of Caucasians are homozygous for a valine to methionine substitution ($Val^{158}Met$) that causes the enzyme to be 4 to 5 fold less effective at methylating catechol substrates in vitro [14].

The sex hormone binding globulin (SHBG) gene encodes for a plasma glycoprotein with a high binding affinity for estradiol and testosterone [15]. A variant in the gene with an aspartic acid to asparagine substitution at codon 327 ($Asp^{327}Asn$) creates an additional site for N-linked glycosylation [16,17] which may increase circulating SHBG levels and reduce circulating bioavailable estradiol levels by increasing the amount bound to SHBG [18].

Although several studies have explored the relationship between these polymorphisms and sex hormone levels in healthy women [18,19], little is known about these associations in breast cancer patients. Therefore, this study examined the associations between genetic polymorphisms in the *CYP17*, *COMT*, *CYP1B1*, and *SHBG* genes with serum levels of total estrone, total estradiol, free estradiol, and estrogen precursor hormones in a cohort of post-menopausal breast cancer patients enrolled in the Health, Eating, Activity and Lifestyle (HEAL) study.

Methods

Study Subjects and Recruitment

The HEAL study is a population-based, multi-center, multi-ethnic prospective cohort study of 1183 breast cancer survivors who are being followed to determine whether weight, physical activity, diet, sex hormones, other exposures and genetic susceptibility polymorphisms affect breast cancer prognosis. Women were recruited into the HEAL study through Surveillance, Epidemiology, End Results (SEER) registries in New Mexico, Los Angeles County (CA), and Western Washington. Details of the aims, study design, and recruitment procedures have been published previously [20].

Briefly, in New Mexico, we recruited 615 women, aged 18 years or older, diagnosed with *in situ* to Stage IIIA breast cancer between July 1996 and March 1999, and living in Bernalillo, Sante Fe, Sandoval, Valencia, or Taos Counties. In Western Washington, we recruited 202 women, between the ages of 40 and 64 years, diagnosed with *in situ* to Stage IIIA breast cancer between September 1997 and September 1998, and living in King, Pierce, or Snohomish Counties. In Los Angeles County, we recruited 366 African-American women aged 35–64 with stage 0 to IIIA primary breast cancer diagnosed between May 1995 and May 1998, who had participated in the Los Angeles portion of the Women's Contraceptive and Reproductive Experiences (CARE) Study [21], a case-control study of invasive breast cancer, or who had participated in a parallel case-control study of *in situ* breast cancer [22].

Participants completed in-person interviews at baseline (within their first year after diagnosis; on average 7.5 months post diagnosis) and at 24-months after the baseline visit (within their third year after diagnosis; on average 31.5 months post diagnosis). Standardized questionnaires collected information on health and medication history, reproductive history, family history of breast and other specific cancers, smoking status, history of oral contraceptive and hormone replacement therapy use, selected demographic data. Post-menopausal status was determined at the 24-month follow-up interview and was defined as having either natural menopause with no periods for at least year or surgical menopause with bilateral oophorectomy.

Of the 1183 women who completed the baseline interview, a total of 240 women did not return for a follow-up visit within three years after diagnosis. Reasons for non-participation were death (44), too ill (2), refusal (104), spouse would not permit contact (1), moved from the study area (16), unable to contact (17), or unable to locate (55).

For the present analysis, we excluded women who did not complete the 24-month interview (240); developed recurrent or new cancer following baseline (84); were pre-menopausal (163),; were taking hormone therapy (other than tamoxifen) at the time of blood draw (35); were missing stage (1), genotyping (93) or hormone (41) data; or were of a race other than white, Hispanic, or African-American (12). An additional 45 women were excluded for unknown menopausal status or hormone levels inconsistent with being post-menopausal. We further excluded another 105 subjects who were not aged 40–64 at baseline in order to use a consistent age range for each study site; hormone levels for women outside this age limit

were different compared to the women within the study age range. The study reported here is based on the remaining 366 postmenopausal women.

Written informed consent was obtained from each subject. We obtained Institutional Review Board approval at each participating center, in accord with an assurance filed with and approved by the U.S. Department of Health and Human Services.

Hormone Assays

A 30-mL 12-hour fasting blood sample was collected for all patients at the 24-month follow-up interview. Blood was processed within 3 hours of collection; serum was stored in 1.8-ml aliquot tubes at −70 to −80 C.

For Californian participants, all hormone assays except testosterone were performed at the Reproductive and Endocrine Research Laboratory at the University of Southern California. Testosterone assays were performed in the Aging and Genetic Epidemiology (AGE) Program Laboratory at the University of New Mexico. For the other two sites (Washington and New Mexico), estrone and estradiol were assayed at Quest Diagnostics (San Juan Capistrano, CA) and SHBG, testosterone and DHEAS assays were conducted in the AGE laboratory. All samples were randomly assigned to assay batches and were randomly ordered within each batch. Laboratory personnel performing the assays were blinded to patient identity and personal characteristics.

Estrone and estradiol assay methods consisted of organic solvent extraction, followed by celite column partition chromatography before quantification by radioimmunoassay (RIA) with sensitivities of 10 and 2 pg/mL, respectively. Testosterone was determined using a RIA kit (Diagnostic Products Corp, Los Angeles, CA) with a sensitivity of sensitivity of 40 pg/mL. SHBG levels were measured with the Radim RIA quantitation SHBG Kit (sensitivity of 6 nmol/L; Wien Laboratories, Succasunna, NJ). DHEAS concentrations were determined using a DHEAS RIA kit (sensitivity of 1.1 μg/dL; Diagnostic Products Corp).

Intra-assay variability was assessed in a reduced randomly selected sample for all hormones. The coefficients of variation (CV) were calculated to test the assay variability. The CV was estimated by the standard deviation of the difference of replicated measures divided by the mean of the two measures. For California samples, 24 blinded blood samples were randomly selected for hormone assay repeats. The intra-assay CVs for estrone, estradiol, and SHBG were 26.2%, 15.4% and 9.3%, respectively. For New Mexico and Washington samples, assays were done in batches, and duplicate aliquots of ten randomly selected participant samples were assayed per bath. The intra-assay CVs for estrone, estradiol, SHBG, and DHEAS were 29.1%, 28.8% 3.8% and 4.6%, respectively. The intra-assay CV for testosterone was 12.0% (done for all centers at the AGE laboratory). These CVs are consistent to those observed in other studies using similar methods for serum concentration of sex hormones [23,24].

Genotype Assessment

For the Washington and California subjects, DNA was extracted from buffy coat fractions using Phenol/Chloroform methods [25]. The *CYP17*, *CYP1B1*, *COMT*, and *SHBG* genotyping were performed at Albany Molecular Research in Bothell, Washington, using the Taqman allelic discrimination method with the ABI 7700 Sequence Detection System (Applied Biosystems, Foster City, CA), which has been described [26,27]. Briefly, the assays measure fluorescent intensity released from allele-specific fluorogenic probes following specific PCR amplification of the genomic flanking regions of the SNP of interest. Probes (see Table 1) were 3'-labeled with the TAMRA quencher dye, and wildtype and variant probes were 5'-labeled with 6-FAM and VIC reporter dyes, respectively (Integrated

DNA Technologies, Coralville, IA). *CYP17* and *CYP1B1* probes were complementary to their corresponding sense strands, whereas the *COMT* probes were complementary to the antisense strand. We included 10% replica samples and genotype concordance was 100%.

For New Mexico subjects, DNA was isolated from buffy coats using the Puregene DNA isolation kit from Gentra Systems (Minneapolis, MN) at the Protein Chemistry Laboratory at the UNM Health Science Center (Albuquerque, NM). The *CYP17* T/C SNP was assessed using a PCR/MspA-I restriction enzyme procedure established by Feigelson et al. [9], using 5'-cattcgcacctctggagtc-3' and 5'-ggctcttggggtacttg-3' primers. *CYP1B1* was analyzed using a PCR/Eco 571 restriction enzyme procedure established by Bailey et al. [10], using primers 5'-gtggtttttgtcaaccagtgg-3' and 5'gcctcttgcttcttattggca-3'. *COMT* genotype was determined using a PCR/Nla-III restriction enzyme procedure [28] using the primers 5'-tactgtggctactcagctgtgc-3' and 5'-gtgaacgtggtgtgaacacc-3'. We conducted a round-robin to check genotyping methods across sites. All round-robin samples were tested at an independent lab and genotype concordance was 100%. Genotyping was not performed for the *SHBG* gene among subjects from New Mexico. Because only 2 Hispanic women had *SHBG* genotyping at the other two sites, these values were excluded from the SHBG analyses.

Statistical Analysis

Free (non-SHBG bound) estradiol and testosterone were calculated indirectly from total estradiol or testosterone, respectively, and serum SHBG using a mathematical equation [29]. Using generalized linear regression, we computed geometric mean values and 95% confidence intervals (CIs) for hormone concentrations for the following genotypes: *CYP17* (A1/A1, A1/A2, A2/A2), *CYP1B1* (Leu/Leu, Leu/Val, Val/Val), *COMT* (Val/Val, Val/Met, Met/Met), and *SHBG* (Asp/Asp, Asp/Asn and Asn/Asn). Hormone levels were entered into models as log-transformed variables due to deviations in the original values from the normal distribution; final estimates were exponentiated. For *CYP17*, *CYP1B1* and *COMT*, analyses were performed separately for each genotype to compare hormone levels by heterozygous and homozygous variant genotypes to the homozygous wild-type (reference) using indicator variables. Due to the low frequency of the homozygous variant genotype for *SHBG*, heterozygous and homozygous variants were combined for comparison with the homozygous wildtype. The androgenic hormones (total testosterone, free testosterone, and DHEAS) are presented for *CYP17* and *SHBG* only because *CYP1B1* and *COMT* act on estrogen alone [11]. Each model was assessed for the presence of non-linearity, heteroskedasticity, and possible outliers using normal quantile plots, as well as plots of the studentized residuals versus X values and fitted values. Trend tests were performed by including the genotype in a linear model.

All genotypes were assessed for potential interaction with tamoxifen use at the time of the blood draw (yes, no), body mass index (BMI) (<30, ≥30) and race (non-Hispanic White, Hispanic White, Non-White); results are stratified when appropriate. In addition, the following variables were considered *a priori* as possible confounders of the associations between genotype and hormone levels: study site (Washington, California, New Mexico), age at diagnosis (continuous), BMI (continuous), percent body fat (continuous), current tamoxifen use (yes, no), ever used hormone therapy (yes, no), ever used oral contraceptives (yes, no), number of ovaries remaining (0, 1+), stage at diagnosis (in situ, stage 1, stage 2), ER status (positive, negative, missing), progesterone receptor status (positive, negative, missing), current smoking status (yes, no), currently drink alcohol (yes, no), marital status (married, widowed, divorced, separated, never married), months since last breast cancer treatment (continuous), and additional polymorphisms that preceded the one in question in the biosynthesis or metabolism pathway of estrogen. Final models were adjusted for those variables that were known *a priori* to be related to estrogen levels or those that changed the

coefficient estimates by 10% or more: study site, race, tamoxifen use, number of remaining ovaries, HT use, marital status, BMI, stage, age.

Results

This analysis included 202 non-Hispanic white, 33 Hispanic white, and 131 African-American breast cancer survivors. By study design, the distribution of study subjects by race/ethnicity differed between study sites. Compared to non-Hispanic white women, the African-American women were younger and less likely to drink alcohol or have used tamoxifen and HT (Table 2). Further, a larger proportion of the African-American women were obese (BMI ≥ 30) or diagnosed with more advanced disease. Although slightly younger and less likely to use HT, the Hispanic white women were similar to the non-Hispanic whites with respect to baseline characteristics. The observed variant allele frequencies differed by race/ethnicity for *COMT* (p=0.001), *CYP1B1* (p<0.001) and *SHBG* (p=0.003). There was no meaningful racial/ethnic difference in the genotype distribution for *CYP17* (p=0.89). Allele frequencies for all genes of interest were in Hardy-Weinberg equilibrium within each racial/ethnic group.

Table 3 shows the mean (geometric) serum hormone levels approximately 24 months following diagnosis by race/ethnicity. Compared to non-Hispanic whites, Hispanic women had significantly lower total estrone (p <0.0001), total testosterone (p<0.0001) and free testosterone (p=0.002); the opposite was found for African-American women who had the highest levels for those hormones (estrone, p <0.0001; testosterone, p <0.0001; free testosterone, p<0.0001) as well as SHBG (p=0.02). There were no differences by race/ethnicity for total estradiol, free estradiol, and DHEAS.

The associations between the *CYP17* polymorphism and several hormones were modified by race/ethnicity (Table 4). Because mean hormone levels did not differ for women with the A1/A2 heterozygous and A2/A2 homozygous variant genotypes, the two groups were combined. Hispanic women with the variant genotype had androgen levels 46–87% higher than those carrying the wildtype alleles. In contrast, androgen levels were 13–20% lower among those with the variant type in non-Hispanic white individuals. Compared to the wildtype, non-Hispanic white women with the variant genotype had lower estrone whereas a slight increase was observed for African-American group (p for interaction = 0.03). Among African-American women, there were no meaningful differences in circulating hormone levels by *CYP17* genotype.

Race/ethnicity was not an important effect modifier for the remaining genes (data not shown). We found little association between serum estrone, estradiol, free estradiol, or SHBG and the *CYP1B1* or *COMT* genotypes (data not shown). We observed an interaction between current tamoxifen use and the *SHBG* genotype for several of the hormones (Table 5). Among women currently using tamoxifen, those with at least one variant allele of the *SHBG* polymorphism had mean serum SHBG and free testosterone concentrations that were 78% higher and 39% lower, respectively, than those with the wildtype. In contrast, for women not using tamoxifen, there was no difference in serum SHBG or free testosterone levels by *SHBG* genotype. However, free estradiol was elevated among women with the variant compared to the wildtype *SHBG* genotype only among women not taking tamoxifen.

Discussion

We found little evidence that polymorphisms for several genes involved with estrogen and androgen metabolism (*CYP17, CYP1B1, COMT or SHBG*) were associated with serum sex hormone levels among a population of postmenopausal breast cancer survivors. However,

we did observe associations for *CYP17* and *SHBG* with Hispanic ethnicity and current tamoxifen use, respectively. Non-Hispanic white women who were heterozygous or homozygous for the variant allele of the *CYP17* genotype had lower estrone, total testosterone, free testosterone, and DHEAS concentrations compared to women homozygous for the wildtype allele. This finding is contradictory to the hypothesized effect which predicted higher hormone levels for individuals with the variant genotype. Among Hispanic whites, the observed effect was in the expected direction with increased estrone and androgen concentrations noted for the variant allele.

Our results for *CYP17* add to the growing number of studies reporting little or no association for genetic control by this particular SNP and hormone levels [18,19,30–36]. Similarly, there is little evidence that circulating hormone levels vary substantially by the *CYP1B1* or *COMT* genotypes [18,19,35,36]; however, the relationship with urinary metabolites of estrogen has been more consistent with higher concentrations observed for the variant genotypes [19,37]. One reason for the lack of an observed effect may be due to the limited ability of most previous studies to look at polymorphisms for several genes or SNPs jointly. For instance, it has been suggested that the functional relevance of *CYP1B1* is dependent on other polymorphisms of the same gene [38]. Similarly, hormone metabolism is a complex biological pathway and it is more likely that disruptions at multiple points alter hormone levels.

Some [18,39], but not all [40], previous studies of the *SHBG* Asp^{327} Asn polymorphism in postmenopausal healthy women have reported higher circulating SHBG levels in carriers of the Asn variant. In our study of breast cancer patients, this finding was restricted to women taking tamoxifen at the time of the blood draw. Among the non-users of tamoxifen, we found no difference in serum SHBG by genotype, but did observe a rise in free estradiol among those with the variant allele which was not expected. The study by Haiman *et al.* which reported no association for the Asp^{327} Asn genotype alone, found lowered serum SHBG levels for women with the wildtype Asp genotype in combination with a repeat polymorphism in the promoter of the *SHBG* gene. This suggests that the function of one polymorphism may depend on the presence of others [40]. However, our subgroup findings for *CYP17* and *SHBG* may also be due to chance given our small numbers and making multiple statistical comparisons [41].

One strength of this study is the inclusion of a multi-ethnic cohort of women with large numbers of non-Hispanic white women and African-Americans. The allele frequencies of many genotypes differ by race or ethnicity and it is probable that particular alleles are linked to other important polymorphisms among particular groups. To our knowledge, this is the only study reporting associations between these specific genotypes and hormone concentrations among a population of breast cancer survivors. Because of the relationship between breast cancer and estrogen, this is a population where estrogen levels are important to study. It is possible that the relationship in patients is different compared to healthy controls. In order to reduce the influence of treatment, hormone concentrations were measured approximately two years following diagnosis. Half of the women in this study were currently taking tamoxifen which can alter estrogen levels. However, with the exception of the SHBG gene, we did not find meaningful genetic associations when both tamoxifen users and non-users were investigated separately.

This study has several limitations. This study was too small to perform stratified analyses for multiple factors simultaneously. We were also unable to assess the joint effects of multiple genes involved with the sex hormone metabolic pathways. We did not have measurements of estrogen metabolites which may be more strongly influenced by *COMT* and *CYP1B1* genotypes. It is also possible that we did not measure the functionally relevant

polymorphisms in the selected genes [8,42]. The coefficients of variation for the estrogen assay were fairly high, likely due to the difficulty of measuring estrogens at low levels in postmenopausal women [20]. These high CVs may be one reason we did not observe strong associations between the genotypes and estrogens.

In conclusion, this study offers little support that polymorphisms in genes involved with sex hormone metabolism directly determine levels of circulating hormones. To understand better the functional relevance of polymorphisms in hormone metabolism and potential consequences regarding risk of cancer and other hormone-dependent diseases, future studies should be large enough to evaluate the independent and joint effects of polymorphisms in genes at each step of the sex hormone metabolic pathway. Future investigations should include both patients and healthy controls to understand whether the functional relevance of the genes under study is different in these two populations. Another issue to address in studies with breast cancer patients is the potential interaction between genotype and use of aromatase inhibitors on sex hormones, since women with ER positive tumors will most likely be treated with these drugs in the future.

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Table 1

Primer and probe sequences used for genotyping of CYP17, CYP1B1, COMT and SHBG genes.

Gene	Primer sequence		Probe sequence	
CYP17	Forward	GGCCTCCTTGTGCCCTAGAGT	Wild-type	VIC-TACTCCAC**T**GCTGTCTATCTTGCCTGC**C**-TAMRA
	Reverse	CCACGAGCTCCCACATGGT	Variant	6FAM-TACTCCAC**C**GCTGTCTATCTTGCCTGC-TAMRA
CYP1B1	Forward	GCTACCACATTCCCAAGGACACT	Wild-type	VIC-TCATGACCCA**G**TGAAGTGGCCT-TAMRA
	Reverse	GTCCAAGAATCGAGCTGGATCA	Variant	6FAM-TCATGACCCA**C**TGAAGTGGCCT-TAMRA
COMT	Forward	GAGGCTCATCACCATCGAGATC	Wild-type	VIC-TGTCCTTCA**C**GCCA**AGCGAAATCC**-TAMRA
	Reverse	GTCTGACAACGGGTCAGGCAT	Variant	6FAM-TGTCCTTCA**T**GCCA**GCGAAATCCA**-TAMRA
SHBG	Forward	CGAGCCACCTTAATGCTCTAATGCC	Wild-type	VIC-CCTCCCTCTAGGAGAA**G**ACTCTTCCAC-TAMRA
	Reverse	CCAGCCTCTGACCTTGTGCCC	Variant	6FAM-CCTCCCTCTAGGAGAA**A**ACTCTTCCAC-TAMRA

Table 2

Selected characteristics of 366[a] postmenopausal breast cancer survivors, stratified by race and ethnicity.

	Non-Hispanic White, n=202 N (%)	Hispanic White, n=33 N (%)	African-American, n=131 N (%)	P value[b]
Age at diagnosis (years)				
40–54	82 (41)	17 (51)	73 (56)	0.02
55–60	120 (59)	16 (49)	58 (44)	
Site				
Washington	77 (38)	2 (6)	1 (1)	n/a
New Mexico	125 (62)	31 (94)	0 (0)	
Southern California	0 (0)	0 (0)	130 (99)	
Tamoxifen use at blood draw				
No	96 (47)	14 (42)	75 (57)	0.14
Yes	106 (53)	19 (58)	56 (43)	
Stage				
In situ	40 (20)	5 (15)	31 (24)	0.002
Local	119 (59)	20 (61)	50 (38)	
Regional	43 (21)	8 (24.)	50 (38)	
Ovaries remaining				
None	38 (19)	6 (18)	24 (18)	0.99
At least 1	164 (81)	27 (82)	106 (82)	
Ever used HRT				
Never	59 (29)	13 (39)	86 (66)	<0.001
Ever	142 (71)	20 (61)	45 (34)	
Body Mass Index, kg/m^2				
< 25	91 (45)	16 (48)	30 (23)	<0.001
25–29.9	62 (31)	11 (33)	36 (27)	
30+	49 (24)	6 (18)	65 (50)	
Marital Status				
Married	134 (67)	22 (69)	50 (38)	<0.001[c]
Widowed	15 (7)	5 (16)	22 (17)	
Divorced/separated	40 (20)	4 (12)	50 (38)	
Never married	12 (6)	1 (3)	9 (7)	
Smoking Status				
Never	95 (47)	17 (52)	61 (47)	0.75
Former	84 (42)	12 (36)	49 (37)	
Current	23 (12)	4 (12)	21 (16)	
Alcohol in last year				
No	45 (22)	8 (24)	77 (59)	<0.001
Yes	157 (78)	25 (76)	54 (41)	
CYP17				
A1/A1 (wild-type)	73 (36)	11 (33)	52 (40)	0.89

	Non-Hispanic White, n=202 N (%)	Hispanic White, n=33 N (%)	African-American, n=131 N (%)	P value[b]
A1/A2	102 (50)	16 (49)	61 (46)	
A2/A2	27 (13)	6 (18)	18 (14)	
CYP1B1				
Val/Val (wild-type)	53 (26)	9 (27)	81 (62)	<0.001
Val/Leu	111 (55)	11 (33)	42 (32)	
Leu/Leu	38 (19)	13 (40)	8 (6)	
COMT				
Val/Val (wild-type)	51 (25)	12 (36)	55 (42)	0.001
Val/Met	92 (46)	13 (39)	61 (46)	
Met/Met	59 (29)	8 (25)	15 (12)	
SHBG gene[d]				
Asp/Asp (wild-type)	61 (79)	n/a	123 (94)	0.003
Asp/Asn or Asn/Asn	16 (21)	n/a	8 (6)	

[a] A few women had missing values for several variables so all subgroups do not add to the total n for the specific racial/ethnic groups.

[b] χ^2 test for differences in characteristics across race/ethnicity.

[c] Fisher's exact test used because of small cell sizes.

[d] The SHBG polymorphism was measured not measured in the 156 women from New Mexico; 2 remaining non-Hispanic women from the other sites were eliminated from analyses of the SHBG genotype.

Table 3

Geometric means and 95% confidence intervals for serum hormone concentrations approximately 24 months following a breast cancer diagnosis, stratified by race and ethnicity.

	Non-Hispanic White, mean (95% CI)	Hispanic White, mean (95% CI)	African-American, mean (95% CI)
Estrone (pg/mL)	21.3 (19.6, 23.2)	16.4 (13.2, 20.3)[a]	31.4 (29.2, 33.8)[a]
Estradiol (pg/mL)	14.1 (12.6, 15.7)	13.5 (10.7–17.0)	14.0 (13.0, 15.2)
Free estradiol (pg/mL)	0.34 (0.30, 0.38)	0.30 (0.24–0.40)	0.31 (0.28, 0.34)
SHBG (nmol/L)	46.5 (43.1, 50.2)	51.3 (41.3, 63.7)	53.8 (49.4, 58.6)[a]
Testosterone (pg/mL)	172 (156, 190)	138 (102, 186)[a]	241 (217, 269)[a]
Free Testosterone (pg/mL)	3.12 (2.80, 3.47)	2.38 (1.70, 3.33)[a]	4.06 (3.61, 4.57)[a]
DHEAS (ug/dL)	58.2 (53.02, 63.8)	51.3 (37.4, 70.2)	65.01 (57.3, 73.8)

[a] $p \leq 0.05$ using ANOVA, comparing mean hormone levels to non-Hispanic white women

Table 4

Adjusted[a] geometric means of sex hormones by CYP17 genotype in postmenopausal breast cancer survivors, stratified by race and ethnicity.

	A1/A1	A1/A2 and A2/A2	
	Geometric Mean (95% CI)	**Geometric Mean (95% CI)**	**Interaction p-value[b]**
Non-Hispanic White			
N	70	127	
Estrone	21.6 (18.7, 24.8)	18.0 (15.5, 21.0)[c]	
Estradiol	8.7 (7.3, 10.4)	8.5 (7.2, 10.1)	
Free estradiol	0.23 (0.19 0.28)	0.23 (0.19, 0.27)	
SHBG	31.4 (27.4, 36.0)	30.6 (26.9, 34.7)	
Testosterone	240 (203, 283)	191 (164, 223)[c]	
Free testosterone	5.3 (4.5, 6.2)	4.3 (3.7, 5.0)[c]	
DHEAS	60.8 (51.4, 71.8)	52.6 (45.4, 60.9)[d]	
Hispanic White			
N	10	20	
Estrone	14.6 (9.8, 21.7)	15.8 (11.8, 21.0)	0.31
Estradiol	9.8 (6.5, 14.7)	6.7 (5.1–8.9)	0.20
Free estradiol	0.25 (0.17–0.39)	0.19 (0.14–0.26)	0.31
SHBG	35.0 (27.9, 43.9)	27.4 (22.2, 33.8)	0.16
Testosterone	145 (84, 248)	240 (182, 314)[c]	0.02
Free testosterone	3.0 (1.7, 5.3)	5.6 (4.2, 7.5)[c]	0.01
DHEAS	43.7 (30.1, 63.4)	63.8 (46.5, 87.5)[d]	0.04
African-American			
N	51	78	
Estrone	35.1 (28.6, 43.0)	37.6 (32.1, 44.1)	0.03
Estradiol	34.1 (27.8, 41.8)	33.0 (28.0, 39.0)	0.97
Free estradiol	0.61 (0.49, 0.76)	0.58 (0.49, 0.69)	0.71
SHBG	107 (89, 131)	117 (102, 137)	0.23
Testosterone	160 (121, 214)	173 (142, 211)	0.05
Free testosterone	1.9 (1.4, 2.6)	2.0 (1.6, 2.4)	0.13
DHEAS	74.0 (56.2, 97.4)	64.9 (53.0, 79.3)	0.93

[a] Adjusted for study site, current tamoxifen use, number of remaining ovaries, ever used HRT, marital status, stage, age at diagnosis, and body mass index.

[b] Comparing mean hormone levels by genotype to non-Hispanic white women

[c] $p \leq 0.05$, comparing mean hormone levels of the variant (A1/A2 or A2/A2) to the wildtype (A1/A1) genotype within each race/ethnicity group

[d] $p \leq 0.10$, comparing mean hormone levels of the variant (A1/A2 or A2/A2) to the wildtype (A1/A1) genotype within each race/ethnicity group

Table 5

Adjusted[a] geometric means of various sex hormones for the SHBG genotype in postmenopausal breast cancer survivors, stratified by tamoxifen status.

	Asp/Asp	Asp/Asn or Asn/Asn	
	Geometric Mean (95% CI)	**Geometric Mean (95% CI)**	**Interaction p-value[b]**
Not using tamoxifen			
N	94	11	
Estrone	27.3 (25.2, 29.6)	26.8 (22.1, 32.6)	
Estradiol	11.1 (10.2, 12.1)	18.9 (14.9, 23.9)	
Free estradiol	0.27 (0.25, 0.29)	0.46 (0.36, 0.59)[c]	
SHBG	44.2 (41.0, 47.8)	46.8 (35.4, 61.9)	
Testosterone	234 (213, 258)	227 (166, 313)	
Free testosterone	4.4 (3.9, 4.8)	4.0 (2.9, 5.5)	
DHEAS	64.8 (58.1, 72.3)	79.7 (56.0, 113.4)	
Using tamoxifen			
N	88	13	
Estrone	29.6 (27.7, 31.6)	25.2 (18.0, 35.3)	0.41
Estradiol	13.3 (12.4, 14.3)	17.8 (13.3, 23.9)	0.10
Free estradiol	0.31 (0.29, 0.34)	0.35 (0.27, 0.45)	0.008
SHBG	50.8 (47.9, 53.8)	90.8 (77.5, 106.2)[c]	0.006
Testosterone	236 (219, 255)	195 (144, 262)	0.47
Free testosterone	4.1 (3.8, 4.5)	2.5 (1.9, 3.4)[c]	0.04
DHEAS	69.5 (63.2, 76.5)	59.5 (39.0, 90.7)	0.17

[a] Adjusted for study site, race, ovaries remaining, ever HRT use, marital status, stage, age at diagnosis, and BMI.

[b] Comparing mean hormone levels by Tamoxifen use.

[c] $p \leq 0.05$, comparing mean hormone levels for the variant (Asp/Asn or Asn/Asn) and the wildtype (Asp/Asp) genotype within each race/ethnicity group.