Association of *CYP17*, *CYP19*, *CYP1B1*, and *COMT* Polymorphisms with Serum and Urinary Sex Hormone Concentrations in Postmenopausal Women

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Abstract

Women with high circulating estrogen concentrations have an increased risk of breast cancer; thus, it is important to understand factors, including genetic variability, that influence estrogen concentrations. Several genetic polymorphisms that may influence sex hormone concentrations have been identified, including *CYP17* (5′-untranslated region T→C), *CYP19* [intron 4 $(TTTA)_{n = 7–13}$ and a 3-bp deletion (−3)], *CYP1B1* (Val^{432}Leu), and *COMT* (Val$^{108/158}$Met). We examined associations between these polymorphisms and serum concentrations of estrogens, androgens, and sex hormone-binding globulin and urinary concentrations of 2- and 16α-hydroxyestrone in 171 postmenopausal women, using data from the prerandomization visit of an exercise clinical trial. Participants were sedentary, not taking hormone therapy, and had a body mass index >24.0. Compared with noncarriers, women carrying two *CYP19* 7r(−3) alleles had 26% lower estrone ($P < 0.001$), 19% lower estradiol ($P = 0.01$), 23% lower free estradiol ($P = 0.01$), and 22% higher sex hormone-binding globulin concentrations ($P = 0.06$). Compared with noncarriers, women carrying at least one *CYP19* 8r allele had 20% higher estrone ($P = 0.003$), 18% higher estradiol ($P = 0.02$), and 21% higher free estradiol concentrations ($P = 0.01$). Women with the *COMT* Met/Met genotype had 28% higher 2-hydroxyestrone ($P = 0.08$) and 31% higher 16α-hydroxyestrone concentrations ($P = 0.02$), compared with Val/Val women. Few associations were found for *CYP17* and *CYP1B1* or with serum androgen concentrations. This study provides further evidence that genetic variation may appreciably alter sex hormone concentrations in postmenopausal women not taking hormone therapy.

Introduction

Postmenopausal women with high circulating estrogen concentrations have an increased risk of developing breast cancer (1, 2). Thus, it is important to identify factors, including environmental and genetic variability, that may alter estrogen concentrations. One such factor that has been identified is obesity; overweight and obese women have higher estrogen concentrations and an increased breast cancer risk compared with lean and normal weight women (3–5). Several genetic polymorphisms that may influence estrogen concentrations have been identified in genes involved in estrogen biosynthesis (*e.g.*, *CYP17* and *CYP19*) and estrogen metabolism (*e.g.*, *CYP1B1* and *COMT*; Fig. 1). Polymorphisms in these genes have been associated with increased breast cancer risk in some populations, but not others (6–8).

The *CYP17* gene codes for cytochrome p450C17α, which ultimately catalyzes the conversion of 17-hydroxypregnenolone and 17-hydroxyprogesterone to dehydroepiandrosterone (DHEA) and androstenedione, respectively. A polymorphism identified in the 5′-untranslated region consists of a T→C substitution at nucleotide 34 (denoted A1/A2), which results in an additional Sp1-type promoter site (9). The A2/A2 genotype has been associated with somewhat higher serum estrone and estradiol concentrations compared with the A1/A1 genotype among 469 postmenopausal women not taking hormone therapy (10, 11). Other studies (n = 114 and 55, respectively) have reported no association (12, 13).

The *CYP19* gene encodes aromatase, an enzyme that converts androstenedione and testosterone to estrone and estradiol, respectively. It has a tetranucleotide repeat polymorphism $(TTTA)_{n = 7–13}$ in intron 4, about 80 bp downstream of exon 4 (14, 15), with the 7 and 11 repeats (11r) being most common (14). There is also a 3-bp deletion 50 bp upstream of the repeat (16). The deletion is found in those with 7 repeats, generating 2 alleles: 7 repeats with the 3-bp deletion [7r(-3)]; and 7 repeats without the deletion (7r). A study of 618 postmenopausal women reported that those carrying the 8-repeat allele (8r) had significantly higher serum estrone, estradiol, and androstenedione concentrations, whereas those carrying either the 7r or 7r(−3) alleles had significantly lower estrone and estradiol concentrations (17). In 85 postmenopausal endometrial cancer

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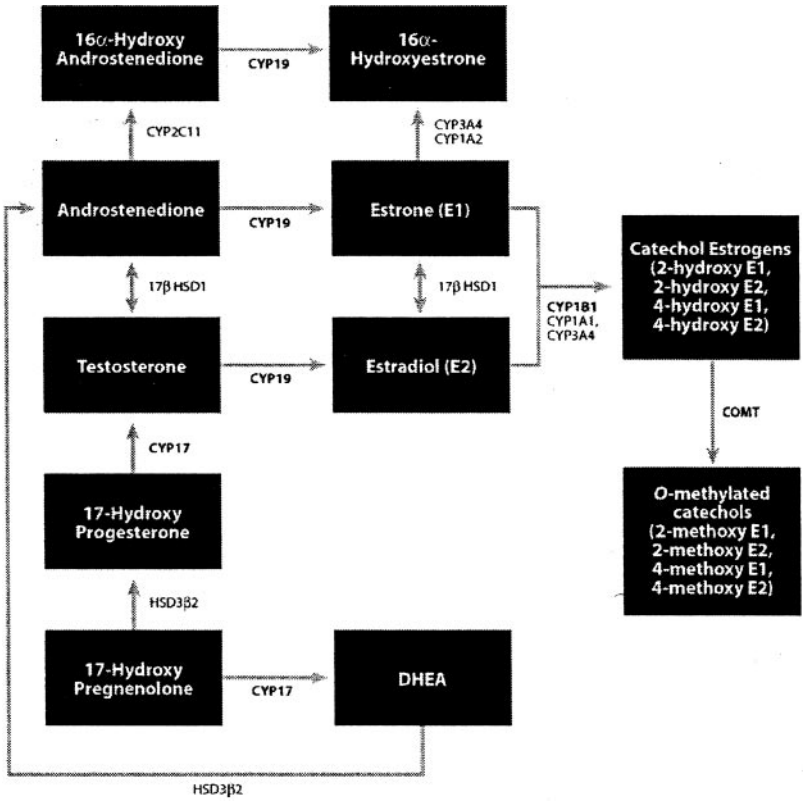

Fig. 1. The biosynthesis and metabolism of estrogen.

patients, the 12-repeat allele was modestly associated with higher estradiol and testosterone levels (18). However, Probst-Hensch *et al.* (19) found no association between repeat number and the estrone/androstenedione ratio in 54 African-American women.

The *CYP1B1* gene codes for an enzyme that catalyzes the addition of a 2- and 4-hydroxyl group to estrone and estradiol; it can carry a mutation that results in a valine/leucine (Val/Leu) substitution at codon 432 (20, 21). The variant protein may have two to three times greater activity than the wild type *in vitro* (22–25); however, the leucine substitution may only affect protein activity in the presence of other *CYP1B1* polymorphisms (25). Among 456 postmenopausal women, those with the Val/Val genotype had significantly lower estradiol levels, a substrate of *CYP1B1*, compared with other genotypes (26), which is consistent with *in vitro* results.

The *COMT* gene encodes catechol-*O*-methyl-transferase, which converts catechol estrogens into inactive metabolites (see Fig. 1) (27). A valine/methionine substitution ($Val^{108/158}Met$) causes the protein to become 2–3-fold less active *in vitro* (28, 29). To our knowledge, no studies have examined the association between *COMT* genotype and sex hormone levels in postmenopausal women; however, the Met allele has been associated with an increased risk of breast cancer in premenopausal and postmenopausal women (8).

This study examines associations between genetic polymorphisms in the *CYP17, CYP19, CYP1B1,* and *COMT* genes and concentrations of various sex hormones in postmenopausal, overweight, sedentary women not taking hormone therapy. Such information will be valuable for establishing the possible functional relevance of these polymorphisms and will enhance our understanding of genetic influences on sex hormone biosynthesis and metabolism in this population. Furthermore, this information may elucidate mechanisms through which genetic variation modifies breast cancer risk.

Materials and Methods

Overview of the Study. Subjects are from the Physical Activity for Total Health Study, which has been described in detail elsewhere (30). Briefly, the study was designed to investigate the effect of a year long moderate intensity exercise intervention *versus* stretching control in 173 postmenopausal women on various hormone endpoints and, secondarily, on changes in body mass index, adiposity, fat distribution, and immune function. Women were ages 50–75 years, sedentary (<60 min per week of moderate to vigorous intensity exercise), and overweight or obese (body mass index ≥ 25.0 or body mass index between 24.0 and <25.0 and percentage of body fat > 33%). Participants resided in the greater Seattle, Washington area. All study procedures, including a written informed consent, were reviewed and approved by the Fred Hutchinson Cancer Research Center Institutional Review Board.

The recruitment process identified potentially eligible women primarily via mass mailings and media advertisements (31). Interested women were screened for eligibility by a phone interview. Major ineligibility criteria included the following: using hormone therapy in the past 6 months; being too physically active; having medical conditions contraindicating moderate to vigorous intensity exercise; having clinical diagnosis of diabetes; and currently using tobacco. Eligible women were scheduled for a screening clinic visit.

Baseline Data Collection. Data used in this study were collected at the prerandomization screening clinic visit. We col-

lected demographic information, medical history, reproductive history, and hormone use history via a self-administered questionnaire. Subjects also completed a food frequency questionnaire (32). We assessed total kilograms of body fat using a DXA whole-body scanner (Hologic QDR 1500; Hologic Inc., Waltham, MA).

Subjects provided a 50-ml sample of blood after fasting for at least 12 h. Blood was processed into serum, plasma, and buffy coats. A spot urine sample also was collected, processed, and stored as aliquots, both unaltered and supplemented with vitamin C (62.5 mg/25 ml urine). All samples were processed within 1 h of collection, aliquoted into 1.8-ml tubes, and stored at –70°C. Date, time of collection, and time since last meal were recorded.

Hormone Assays. Serum hormone assays were performed at the Reproductive Endocrine Research Laboratory (University of Southern California), directed by one of the authors (F. Z. S.). Estradiol, estrone, testosterone, androstenedione, and DHEA were quantified by sensitive and specific radioimmunoassay after organic solvent extraction and Celite column partition chromatography (19, 33). Chromatographic separation of the steroids was achieved by use of different concentrations of toluene in isooctane and ethyl acetate in isooctane. Sex hormone-binding globulin (SHBG) was quantified via an immunometric assay, and dehydroepiandrosterone sulfate (DHEAS) was quantified via a competitive immunometric assay, both using the Immulite Analyzer (Diagnostic Products Corp.). Free estradiol and testosterone were calculated using the measured estradiol and testosterone concentrations, respectively, SHBG concentrations, and an assumed constant for albumin (34, 35). This method has been found to have high validity compared with direct measurement (36).

Urinary estrogen metabolites 2- and 16α-hydroxyestrone were assayed in the Johanna Lampe Laboratory at the Fred Hutchinson Cancer Research Center by one of the authors (C. A.). Concentrations were determined from urine samples supplemented with vitamin C using the commercially available Estramet 2/16 enzyme immunoassay kits (Immunacare Corp., Bethlehem, PA), as described elsewhere (37). Urinary excretion of both metabolites was normalized by urinary creatinine concentrations determined using the Roche Cobas Mira Plus Chemistry Analyzer (Roche Diagnostic Systems, Nutley, NJ), which is based on a kinetic modification of the Jaffe reaction.

Biological samples were batched such that, within each batch, the randomization dates of subjects were similar, the number of intervention and control subjects was approximately equal, and the sample order was random. For quality control, we created serum and urine pools from ineligible subjects (postmenopausal and not taking exogenous estrogens) and placed two specimens of the pooled sample in each batch. Laboratory personnel were blinded to subject and quality control sample identity and randomization status. The intra-assay coefficients of variation (CVs) were $<$10%, and the interassay CVs were $<$12% for all assays except for estrone (intra-assay CV = 12.4%, interassay CV = 17.6%), estradiol (intra-assay CV = 12.4%, interassay CV = 15.8%), 2-hydroxyestrone (interassay CV = 12.2%), and 16α-hydroxyestrone (interassay CV = 15.3%).

Genotyping Assays. DNA was extracted from buffy coat fractions using two phenol:chloroform extractions and a single chloroform extraction; DNA was precipitated using 100% ethanol and a salt solution of sodium acetate. The extraction protocol was conducted at the Core Specimen Processing Laboratory (Fred Hutchinson Cancer Research Center, Seattle, WA).

Table 1 Primers and allele probes used in the genotyping assays

Gene	Forward and reverse primers	Wild-type and variant allele probes
CYP17	5′-gccctttaaaaggcctccttgt-3′	5′-tcttctactccacTgctgtctatct-3′
	5′-ccacgagctcccacatggt-3′	5′-cttctactccacCgctgtctatct-3′
CYP19	5′-ctctggaaaacaactcgaccct-3′	NA[a]
	5′-ggttacagtgagccaaggtcgt-3′	
CYP1B1	5′-cccaaggacactgtggtttttgt-3′	5′-tgaatcatgacccaGtgaagtggccta-3′
	5′-tgttgatgaggccgtccttgt-3′	5′-tgaatcatgacccaCtgaagtggccta-3′
COMT	5′-atcgagatcaaccccgactgt-3′	5′-cttgtccttcaCgccagcgaaat-3′
	5′-agccggccctttttcca-3′	5′-cttgtccttcaTgccagcgaaatc-3′

[a] NA, not applicable.

Genotyping assays were performed at the Center for Ecogenetics and Environmental Health Functional Genomics Laboratory (University of Washington), directed and performed by two of the authors (F. M. F. and P. L. S.). The specific single-nucleotide polymorphisms in the *CYP17*, *CYP1B1*, and *COMT* genes were identified using 5′-nuclease assays performed and analyzed on an ABI PRISM 7700 Sequence Detection System, which uses fluorogenic TaqMan probes (PE Applied Biosystems, Foster City, CA). Probes (Table 1) were 3′-labeled with the TAMRA quencher dye, and wild-type and variant probes were 5′-labeled with 6-FAM and VIC reporter dyes, respectively (Integrated DNA Technologies, Coralville, IA). Probes were complementary to their corresponding sense strands, except for the COMT probes, which were complementary to the antisense strand.

A fluorescence-labeled (5′-FAM) forward primer was used in the PCR that characterized the *CYP19* tetranucleotide repeat and deletion polymorphisms. Specific PCR primers (Table 1) were designed using PrimerExpress software (PE Applied Biosystems) and purchased from MWG Biotech (High Point, NC). After the PCR, the product was diluted in high-performance liquid chromatography water, mixed with Genescan-500 TAMRA size standard loading buffer, loaded on a 5% polyacrylamide gel, and sequenced using an ABI Prism 377 DNA Sequencer (PE Applied Biosystems). Samples were analyzed using GENOTYPER software (PE Applied Biosystems). Aliquots of sample PCR products were sequenced using the dye terminator method to confirm the genotypes.

For quality control purposes, we included a blinded DNA sample, collected at a second prerandomization clinic visit, for 18 subjects. All quality control samples were in perfect concordance with their corresponding samples.

Statistical Analysis. We examined associations between sex hormones and the following genotypes: *CYP17* (A1/A1, A1/A2, or A2/A2); *CYP19* 7r(−3) (no copies, one copy, or two copies); *CYP19* 7r (no copies or one/two copies); *CYP19* 8r (no copies or one/two copies); *CYP19* 11r (no copies, one copy, or two copies); *CYP1B1* (Leu/Leu, Leu/Val, or Val/Val); and *COMT* (Val/Val, Val/Met, or Met/Met). Although some subjects had 9, 10, 12, and 13 *CYP19* repeats, there were too few to analyze these alleles separately (all had allele frequencies of $\leq$3%).

We determined adjusted geometric means and 95% confidence intervals using linear regression on log-transformed hormone concentrations, adjusting for age (linear), kilograms of body fat from DXA (linear), oophorectomy status (no ovaries, had at least part of one ovary, unknown), race (non-Hispanic white, other), ever used hormone therapy (yes, no), ever used herbal hormones (yes, no), marital status (never

Table 2 Baseline demographic characteristics of the study participants

	Subjects (n = 171), mean (SD) or %
Age (yrs)	60.6 (6.6)[a]
Total body fat from DXA (kg)	38.4 (9.0)[a]
Alcohol (g/day)	4.3 (7.9)[a]
Ever used hormone therapy	72 (42.1)[b]
Ever used herbal hormones[c]	12 (7.1)[b]
No ovaries remaining	7 (4.1)[b]
Had hysterectomy	30 (17.5)[b]
Race/ethnicity[d]	
Non-Hispanic white	148 (87.1)[b]
Other	22 (12.9)[b]
Marital status	
Never married	12 (7.0)[b]
Divorced/separated	44 (25.7)[b]
Widowed	18 (10.5)[b]
Married/living with partner	97 (56.7)[b]

[a] Number in parentheses is SD.
[b] Number in parentheses is percentage.
[c] Based on the question "Did you ever take any type of herbal estrogen, progesterone, or testosterone, such as Dong Quai (*Angelica sinenis*), Black Cohash (*Cimicifuga racemosa*), Wild Yam, or other herbal hormones?"
[d] One subject did not report her race/ethnicity.

married, divorced/separated, widowed, married/living with partner), alcohol consumption (<0.5 g/day, 0.5–5 g/day, >5 g/day), and the three other genotypes. In the *CYP17*, *CYP1B1*, and *COMT* analyses, we adjusted only for the allele copy number of *CYP19* 7r(−3), to avoid having highly correlated variables in the model. Adjustment variables were chosen *a priori* to increase precision. Trend tests were determined by including the genotype as a linear term in the model. Results did not differ substantially by race or previous hormone therapy use, so we combined all subjects.

One woman was using vaginal estrogen cream at her blood draw, and one woman had an extremely high SHBG concentration (191 nmol/liter), indicating hormone use. Their data were excluded from the analyses, leaving 171 subjects.

Results

Subjects were, on average, 61 years old and had 38.4 kg of body fat as measured by DXA (Table 2). Over 42% of women had taken hormone therapy in the past, whereas 7% had taken some form of herbal hormones. Eighty-seven percent of subjects were non-Hispanic white. Allele frequencies were in Hardy-Weinberg equilibrium (χ^2, $P > 0.17$ for all alleles) for the subjects overall and within racial/ethnic groups (Table 3). The two most common *CYP19* alleles were the 11r and 7r(−3) alleles. The *CYP19* 7r distributions were significantly different between white and non-white women ($P = 0.001$); no other genotype distributions differed significantly by race.

Women with the *CYP17* A1/A2 and A2/A2 genotypes had 19% and 24% higher SHBG concentrations ($P = 0.02$ for both), respectively, compared with A1/A1 women (Table 4). The A2 allele was modestly associated with increased levels of 2-hydroxyestrone (P-trend = 0.06). This trend was not as strong (P-trend = 0.23) when the 22 non-white women were excluded (data not shown). We found little association between *CYP17* genotype and the other sex hormones.

The *CYP19* 7r and 11r alleles were not significantly associated with hormone concentrations (Table 5). Compared with noncarriers, women with one *CYP19* 7r(−3) allele had 9% lower estrone ($P = 0.06$), 10% lower free estradiol ($P = 0.07$), and 15% higher SHBG concentrations ($P = 0.03$). Similarly, women homozygous for *CYP19* 7r(−3) had 26% lower estrone ($P < 0.001$), 19% lower estradiol ($P = 0.01$), 23% lower free estradiol ($P = 0.01$), and 22% higher SHBG ($P = 0.06$) concentrations. Compared with noncarriers, women with one *CYP19* 7r(−3) allele had 13% higher androstenedione ($P = 0.04$) and 19% higher testosterone concentrations ($P = 0.01$). However, homozygous women [7r(−3)/7r(−3)] did not have significantly different concentrations of these hormones from noncarriers. Women carrying at least one *CYP19* 8r allele had 20% higher estrone ($P = 0.003$), 18% higher estradiol ($P = 0.02$), and 21% higher free estradiol concentrations ($P = 0.01$), compared with noncarriers. The results for all CYP19 alleles did not change substantially when the 22 non-white women were excluded (data not shown).

We found little association between *CYP1B1* genotype and estrogens, androgens, SHBG, and urinary estrogen metabolites (data not shown); results were similar when the 22 non-white women were excluded (data not shown). Women with the *COMT* Met/Met genotype had 28% higher 2-hydroxyestrone ($P = 0.08$) and 31% higher 16α-hydroxyestrone concentrations ($P = 0.02$), compared with Val/Val women (data not shown). However, when the 22 non-white women were excluded, the nearly statistically significant association between COMT genotype and 2-hydroxyestrone became nonsignificant (P-trend = 0.49); the associations with other sex hormones were similar (data not shown). No other hormones were significantly associated with *COMT* genotype (data not shown).

Table 3 Genotype distributions of study participants, stratified by race

Genotype	Non-Hispanic white n (%)	Other[a] n (%)	P^b
CYP17			
A1/A1	53 (35.8)	8 (36.4)	
A1/A2	67 (45.3)	10 (45.4)	0.99
A2/A2	28 (18.9)	4 (18.2)	
CYP19 7r(−3)			
No copies	58 (39.2)	11 (50.0)	
7r(−3)/X	75 (50.7)	10 (45.5)	0.59[c]
7r(−3)/7r(−3)	15 (10.1)	1 (4.5)	
CYP19 7r			
Noncarrier	118 (79.7)	10 (45.4)	0.001[c]
Carrier	30 (20.3)	12 (54.6)	
CYP19 8r			
Noncarrier	115 (77.7)	20 (90.9)	0.26[c]
Carrier	33 (22.3)	2 (9.1)	
CYP19 11r			
No copies	57 (38.5)	12 (54.5)	
11r/X	73 (49.3)	9 (40.9)	0.35[c]
11r/11r	18 (12.2)	1 (4.6)	
CYP1B1			
Val/Val	26 (17.6)	4 (18.2)	
Val/Leu	76 (51.3)	9 (40.9)	0.65[c]
Leu/Leu	46 (31.1)	9 (40.9)	
COMT			
Val/Val	40 (27.0)	9 (40.9)	
Val/Met	78 (52.7)	8 (36.4)	0.29[c]
Met/Met	30 (20.3)	5 (22.7)	

[a] Includes American Indian, Asian/Pacific Islander, African American, Hispanic white, and other.
[b] χ^2 test for differences in genotype across race.
[c] Used Fisher's exact test because of small cell sizes.

Table 4 Adjusted[a] geometric means (95% confidence intervals) of various sex hormones in postmenopausal women, stratified by *CYP17* genotype

	A1/A1 (n = 61)	A1/A2 (n = 78)	A2/A2 (n = 32)	P-trend
Estrone (pg/ml)	42.9 (40.2–45.8)	45.2 (42.2–48.5)	42.4 (37.1–48.5)	0.43
Estradiol (pg/ml)	17.8 (16.7–19.0)	18.6 (17.2–20.0)	17.1 (15.2–19.1)	0.74
Free estradiol (pg/ml)	0.50 (0.46–0.53)	0.49 (0.46–0.53)	0.45 (0.39–0.51)	0.21
SHBG[c] (nmol/liter)	30.9 (27.8–34.4)	37.1[b] (34.1–40.2)	39.0[b] (33.9–44.9)	0.005
2-Hydroxyestrone[d] (ng/mg creatinine)	5.9 (5.1–6.8)	7.1[e] (6.1–8.2)	7.5 (5.8–9.8)	0.06
16α-Hydroxyestrone[d] (ng/mg creatinine)	5.5 (4.7–6.4)	6.0 (5.3–6.7)	6.2 (5.0–7.7)	0.33
Androstenedione (ng/ml)	556 (513–602)	563 (519–612)	541 (446–656)	0.86
Testosterone (pg/ml)	214 (192–238)	218 (200–238)	217 (186–253)	0.84
Free testosterone (pg/ml)	4.9 (4.4–5.4)	4.6 (4.2–5.0)	4.5 (3.9–5.2)	0.33
DHEA (ng/ml)	2.5 (2.2–2.8)	2.2 (2.0–2.5)	2.1 (1.7–2.7)	0.20
DHEAS (μg/dl)	60.4 (50.1–72.9)	56.2 (48.4–65.3)	51.3 (38.5–68.2)	0.32

[a] Adjusted for *CYP19* 7r(−3), *CYP1B1*, *CYP1B1*, *COMT*, age, kilograms of body fat, oophorectomy status, race, ever used hormone therapy, ever used herbal hormones, marital status, and alcohol consumption.
[b] $P \leq 0.05$, compared with A1/A1.
[c] SHBG, sex hormone-binding globulin; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate.
[d] Urinary hormone.
[e] $P \leq 0.10$, compared with A1/A1.

Discussion

The purpose of this study was to determine whether polymorphisms in genes involved in estrogen biosynthesis and metabolism were associated with concentrations of estrogens, estrogen metabolites, androgens, and SHBG in postmenopausal, overweight women not taking hormone therapy. We found evidence that *CYP17*, *CYP19*, and *COMT* genotypes were associated with sex hormones in this population. These findings support the hypothesis that polymorphisms in these genes play a role in determining sex hormone concentrations and are potentially involved cancer etiology.

The *CYP17* A2 allele was associated with increased SHBG concentrations and possibly associated with increased 2-hydroxyestrone levels. One previous study reported that the *CYP17* A2/A2 genotype was associated with a lower 2-hydroxyestrone:16α-hydroxyestrone ratio in 494 premenopausal women (38). However, it is difficult to explain these results because *CYP17* genotype was not associated with intermediary hormones in the pathway, including its direct products, dehydroepiandrosterone and androstenedione. Although it is possible that *CYP17* acts through some other pathway that alters SHBG and estrogen metabolite concentrations, the associations we found may be spurious, given the large number of statistical comparisons in this study and the observation that the modest association with 2-hydroxyestrone disappeared when non-white women were excluded. We found no association between *CYP17* genotype and estrogen concentrations, which is inconsistent with the results of Haiman *et al.* (11). They reported a modest increase in estrone and estradiol with increasing copies of the A2 allele in 469 postmenopausal women who had never taken hormone therapy. When we restricted our analysis to the 99 women who had never taken hormone therapy, we still did not detect an association between *CYP17* genotype and estrogens (data not shown). Two other studies of 114 endometrial cancer patients (13) and 55 women with polycystic ovary syndrome (12) reported no associations. Discrepancies between studies may be due to the differing populations and sample sizes.

We found evidence that the *CYP19* 7r(−3) allele was associated with decreased estrogen and increased SHBG concentrations, whereas the *CYP19* 8r allele was associated with increased estrogen concentrations. Our findings are consistent with those of Haiman *et al.* (17), who reported that, among 443 postmenopausal women who had not used hormones for at least 3 months, those carrying the 7r or 7r(−3) alleles had lower estrone and estradiol concentrations compared with noncarriers. In the same study, women carrying the 8r allele had higher estrone and estradiol concentrations and lower androstenedione concentrations compared with noncarriers. However, two studies reported no association between the 7r or 8r alleles and the estrone:androstenedione ratio in 54 postmenopausal African-American women (19) or estradiol and testosterone in 85 postmenopausal endometrial cancer patients (18). The null results in these latter studies may be due to the small sample sizes or specific subpopulations studied. Despite this, current evidence suggests that the tetranucleotide repeat and/or the deletion polymorphisms are associated with estrogen concentrations in postmenopausal women not taking hormone therapy.

Although no data exist on the functionality of either the *CYP19* tetranucleotide repeat or deletion polymorphisms, both occur in intron 4 near the exon/intron border with exon 4, possibly near a splice site (14, 39). The different genetic variants may lead to alternate splicing patterns and mRNA transcripts, which could ultimately result in a protein with modified activity. Our results, in combination with those of Haiman *et al.* (17), suggest that this polymorphism or one in linkage disequilibrium with it may alter the binding affinity of the CYP19 protein (aromatase) to testosterone and androstenedione

Table 5 Adjusted[a] geometric means (95% confidence intervals) of various sex hormones in postmenopausal women, stratified by *CYP19* (TTTA) repeat and 3-bp deletion alleles

	7r Allele		7r(−3) Allele			
	None (n = 128)	One or two (n = 43)	None (n = 70)	One (n = 85)	Two (n = 16)	P-trend
Estrone (pg/ml)	43.5 (41.1–46.0)	44.9 (41.1–49.1)	47.4 (44.2–50.9)	42.9[b] (40.0–46.1)	35.1[c] (31.0–39.7)	<0.001
Estradiol (pg/ml)	18.1 (17.1–19.2)	17.6 (16.2–19.2)	18.9 (17.5–20.4)	17.9 (16.7–19.1)	15.3[c] (13.3–17.7)	0.03
Free estradiol (pg/ml)	0.48 (0.46–0.52)	0.49 (0.45–0.53)	0.52 (0.48–0.57)	0.47[b] (0.44–0.51)	0.40[c] (0.34–0.48)	0.005
SHBG[d] (nmol/liter)	35.9 (33.6–38.4)	32.6 (28.4–37.4)	32.1 (29.5–34.8)	36.9[c] (33.6–40.6)	39.0[b] (32.8–46.4)	0.01
2-Hydroxyestrone[e] (ng/mg creatinine)	7.0 (6.3–7.8)	5.9 (4.8–7.3)	6.4 (5.4–7.5)	6.8 (6.0–7.8)	7.4 (5.0–11.1)	0.37
16α-Hydroxyestrone[e] (ng/mg creatinine)	6.0 (5.4–6.6)	5.4 (4.4–6.6)	5.4 (4.7–6.2)	6.0 (5.3–6.7)	7.0 (5.2–9.4)	0.09
Androstenedione (pg/ml)	571 (534–610)	514 (457–578)	525 (477–577)	596[c] (550–646)	498 (402–618)	0.54
Testosterone (pg/ml)	219 (205–234)	210 (185–238)	198 (180–218)	235[c] (216–256)	207 (171–249)	0.11
Free testosterone (pg/ml)	4.7 (4.4–5.0)	4.7 (4.2–5.3)	4.5 (4.1–4.8)	4.9 (4.5–5.4)	4.3 (3.4–5.4)	0.61
	8r Allele		11r Allele			
	None (n = 136)	One or two (n = 35)	None (n = 69)	One (n = 83)	Two (n = 19)	P-trend
Estrone (pg/ml)	42.3 (40.2–44.5)	50.6[c] (45.5–56.2)	42.4 (39.3–45.8)	45.5 (42.4–48.9)	42.5 (37.3–48.3)	0.51
Estradiol (pg/ml)	17.4 (16.6–18.3)	20.5[c] (18.0–23.3)	17.5 (16.1–18.9)	18.5 (17.3–19.8)	17.8 (15.4–20.5)	0.53
Free estradiol (pg/ml)	0.47 (0.44–0.49)	0.56[c] (0.49–0.65)	0.47 (0.43–0.52)	0.49 (0.46–0.53)	0.50 (0.43–0.58)	0.45
SHBG (nmol/liter)	35.9 (33.6–38.4)	31.8 (27.8–36.4)	34.6 (31.2–38.3)	36.2 (32.9–40.1)	31.7 (28.7–35.0)	0.74
2-Hydroxyestrone[e] (ng/mg creatinine)	6.9 (6.2–7.6)	6.1 (5.0–7.5)	6.9 (5.9–8.1)	6.6 (5.7–7.6)	6.5 (5.1–8.3)	0.60
16α-Hydroxyestrone[e] (ng/mg creatinine)	6.0 (5.5–6.6)	5.0 (4.1–6.2)	6.0 (5.2–6.9)	5.8 (5.2–6.6)	5.1 (4.1–6.5)	0.36
Androstenedione (pg/ml)	549 (516–584)	585 (508–674)	547 (494–605)	571 (530–616)	531 (435–648)	0.94
Testosterone (pg/ml)	215 (202–228)	223 (188–264)	219 (196–244)	222 (206–240)	187 (154–227)	0.38
Free testosterone (pg/ml)	4.6 (4.3–4.9)	5.0 (4.3–5.8)	4.8 (4.3–5.3)	4.7 (4.4–5.1)	4.3 (3.6–5.2)	0.51

[a] Adjusted for *CYP17*, *CYP1B1*, *COMT*, age, kilograms of body fat, oophorectomy status, race, ever used hormone therapy, ever used herbal hormones, marital status, and alcohol consumption.
[b] $P \leq 0.10$, compared with no copies of that allele.
[c] $P \leq 0.05$, compared with no copies of that allele.
[d] SHBG, sex hormone-binding globulin.
[e] Urinary hormone.

versus 16α-hydroxyandrostenedione. Data suggest that 16α-hydroxyandrostenedione has a separate binding site from that of testosterone and androstenedione but that each hormone inhibits the aromatization of the others (40, 41). Thus, individuals with the 7r(−3) allele may produce proteins with a higher binding affinity for 16α-hydroxyandrostenedione, which would inhibit the binding of androstenedione and testosterone. This would explain the slightly higher 16α-hydroxyestrone (P = 0.12) and the lower estrone and estradiol concentrations associated with the 7r(−3) *CYP19* allele.

We found little evidence to suggest that the *CYP1B1* Val/Leu polymorphism was associated with estrogen concentrations, despite previous observations that the leucine substitution causes the protein to have a 2–3-fold higher activity *in vitro* (22–25). However, Aklillu *et al.* (25) reported that the leucine substitution affects protein activity only in the presence of other *CYP1B1* polymorphisms. Because we did not evaluate these other polymorphisms, we may have misclassified subjects with respect to protein activity and therefore were unable to detect an association. Future studies should consider assaying multiple polymorphisms in the *CYP1B1* gene to address this possible limitation.

Although the *COMT* Val/Met polymorphism was not associated with estrone or estradiol concentrations directly, women homozygous for the Met allele had higher urinary 2-hydroxyestrone and 16α-hydroxyestrone concentrations. The

methionine substitution causes the protein to become 2–3-fold less active *in vitro* (28, 29). Our results suggest that the variant protein may also be less active *in vivo*, at least with respect to 2-hydroxyestrone metabolism, although this result should be interpreted cautiously because the association disappeared when non-white women were excluded. This could be of particular significance because 2-hydroxyestrone is an intermediate for the generation of reactive quinines and semiquinones, which have been implicated in cancer initiation (42). Because *COMT* is involved in estrogen metabolism, it seems possible that there is a feedback loop that may explain the association with 16α-hydroxyestrone; however, we know of no data to support this hypothesis. The observed association could also be spurious, due to multiple statistical comparisons.

This study has several limitations. First, it is unclear whether the *CYP17* and *CYP19* polymorphisms directly affect enzyme function. Further research is needed to explore the functional relevance of these polymorphisms both *in vitro* and *in vivo*. Second, the study population had to meet the stringent eligibility criteria of our exercise intervention trial to be included in the present study. Therefore, caution must be used in generalizing our results to all postmenopausal women, especially those taking hormone therapy. However, the study population was fairly homogenous; high postmenopausal hormone concentrations were seen because all participants were overweight or obese. Although this may have increased the power to detect an association, our results may not be generalizable to lean or normal weight women. Another advantage of this study was the ability to adjust for polymorphisms of other genes in the analysis. Finally, the significant trend tests observed, particularly for the *CYP19* 7r(−3) and *COMT* polymorphisms, add credibility that the associations we observed are not spurious.

Our results suggest that the *CYP17*, *CYP19*, and *COMT* but not *CYP1B1* polymorphisms may alter sex hormone concentrations in some postmenopausal women. This information may help elucidate pathways through which variations in these genes may influence breast cancer etiology. Due to the complex patterns of genotype-phenotype associations, future studies should consider multiple polymorphisms concurrently.

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Association of *CYP17, CYP19, CYP1B1*, and *COMT* Polymorphisms with Serum and Urinary Sex Hormone Concentrations in Postmenopausal Women

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