Genetic Factors in Catechol Estrogen Metabolism in Relation to the Risk of Endometrial Cancer

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Abstract

2-Hydroxylated metabolites of estrogen have been shown to have antiangiogenic effects and inhibit tumor cell proliferation, whereas 4-hydroxylated metabolites have been implicated in carcinogenesis. We examined whether polymorphisms in certain genes involved in estrogen metabolism are associated with endometrial cancer risk in a population-based case-control study with 371 cases and 420 controls. Based on previously published genotype-phenotype correlation studies, we defined variant alleles thought to increase estrogen 2-hydroxylation as presumptively low-risk (*CYP1A1* m1 T6235C and m2 Ile^{462}Val) and those thought to increase estrogen 4-hydroxylation as high-risk (*CYP1A1* m4 Thr^{461}Asn, *CYP1A2* A734C, and *CYP1B1* Leu^{432}Val). Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated using unconditional logistic regression. Carrying at least one *CYP1A1* m1 or m2 variant allele was associated with a decreased risk of endometrial cancer [ORs (95% CIs), 0.64 (0.44-0.93) and 0.54 (0.30-0.99), respectively]. No strong alteration in risk was observed among women with any of the putative high-risk alleles. When *CYP1A1*, *CYP1A2*, and *CYP1B1* genotypes were combined and ranked by the number of putative low-risk genotypes carried, women with four or five low-risk genotypes had a reduced risk of endometrial cancer (OR, 0.29; 95% CI, 0.15-0.56) compared with women with one or none. No appreciable alteration in risk was observed among women carrying two or three low-risk genotypes. Some of our findings are consistent with the hypothesis that increased estrogen 2-hydroxylation is associated with decreased endometrial cancer risk, but replication of these results is required before any firm conclusions can be reached. (Cancer Epidemiol Biomarkers Prev 2005;14(2):357–66)

Introduction

The risk of endometrial cancer is elevated for women in whom levels of estrogens are relatively high, whether the source of estrogen is exogenous (e.g., via hormonal medications) or endogenous (e.g., as a result of obesity; ref. 1). It is plausible that interindividual variation in genes that govern the activity of enzymes involved in estrogen metabolism may play a role in susceptibility to endometrial cancer.

The principal estrogens in women, estradiol and estrone, undergo oxidative metabolism through hydroxylation at various sites, including 2- and 4-hydroxylation, leading to the formation of catechol estrogens [reviewed in Zhu and Conney (2); Fig. 1]. 2-Hydroxylation is the major oxidative pathway, catalyzed mainly by CYP1A2 in the liver (3, 4) and by CYP1A1 in the endometrium (5) and other extrahepatic tissues. In Syrian hamsters, almost 100% of whom develop kidney tumors after exposure to estradiol, 2-hydroxylated estrogens (2-OH estrogens) do not induce tumors (6, 7). The *O*-methylation of 2-hydroxyestradiol (2-OH estradiol) forms 2-methoxyestradiol, which is a potent inhibitor of tumor cell proliferation and has antiangiogenic effects (8, 9). It is currently being evaluated in phase I and II clinical trials of breast and prostate cancers (10).

In contrast to 2-OH estrogens, there are several reasons why 4-OH estrogens might be important in carcinogenesis. Although 4-OH estrogens are formed in much smaller amounts than 2-OH estrogens in the liver (3, 4), 4-OH estradiol may accumulate in target organs, such as the endometrium, when high levels of 2-OH estradiol inhibit the *O*-methylation of 4-OH estradiol (11, 12). In CD-1 mice, 4-OH estradiol more strongly induces endometrial adenocarcinoma than does 2-OH estradiol (13). 4-OH estradiol is as carcinogenic as estradiol in the Syrian hamster kidney (6, 7), and the ratio of estradiol 4- to 2-hydroxylation increases during tumorigenesis (14). This pattern is also observed in human uterine leiomyomata compared with normal myometrium (12). Additionally, 4-OH estradiol (unlike 2-OH estradiol) possesses potent hormonal activity because it activates the estrogen receptor (2). CYP1B1 is the primary enzyme involved in the 4-hydroxylation of estrone and estradiol (15), and it is strongly expressed in the endometrium (5).

The 2- and 4-OH estrogens can be further oxidized to semiquinones and quinones, which can undergo redox cycling, producing reactive oxygen species that may cause oxidative stress, lipid peroxidation, and DNA damage (reviewed in refs. 16, 17). The 4-OH estrogen quinones, in contrast to 2-OH estrogen quinones, form depurinating DNA adducts, which could potentially be involved in tumor initiation, by producing mutations in critical genes (16). The enzyme catechol-*O*-methyltransferase (COMT), which is expressed in various tissues, including the endometrium (18), transforms catechol estrogens into inactive metabolites and prevents them from entering into redox cycling (19). In the Syrian hamster, inhibiting COMT activity results in increased kidney tumorigenesis (20). The quinones can be deactivated by conjugation with glutathione by glutathione *S*-transferases (GST; ref. 21), which are expressed in the endometrium (22, 23).

The genes mentioned above contain several well-characterized polymorphisms. For *CYP1A1*, these include *CYP1A1* m1 (or *Msp*I, T6235C), which has been observed to be associated

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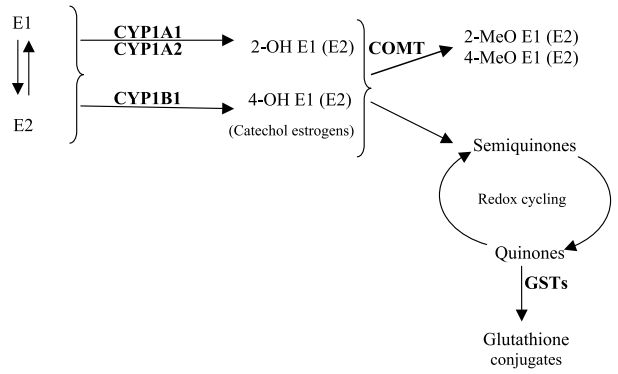

Figure 1. Estrogen catabolism by CYP1A1, CYP1A2, CYP1B1, COMT, and GSTs. *E1,* estrone; *E2,* estradiol; *2-OH E1 (E2),* 2-hydroxyestrone (estradiol); *4-OH E1 (E2),* 4-hydroxyestrone (estradiol); *2-MeO E1 (E2),* 2-methoxyestrone (estradiol); *4-MeO E1 (E2),* 4-methoxyestrone (estradiol).

with a high inducibility phenotype in several (24-28) but not all (29-31) studies; *CYP1A1* m2 (exon 7, A4889G, Ile^{462}Val), which may be associated with increased enzyme activity and inducibility (27, 28, 32-34), although some studies did not observe this (26, 35-37); and *CYP1A1* m4 (exon 7, C4887A, Thr^{461}Asn), which encodes for a protein that has been observed to exhibit *reduced* activity toward estradiol (reported in abstract form; ref. 38), testosterone, and progesterone (36). For a *CYP1A2* A734C substitution in intron 1, two [ref. 39 (an abstract) and ref. 40] of three studies (41) observed that carriage of the C allele was associated with decreased inducibility. *CYP1B1* contains a Leu^{432}Val change (C1294G) in exon 3. Generally, the Val^{432} allele seems to result in increased 4-OH metabolite formation compared with the Leu^{432} allele (42-49). A Val^{158}Met substitution due to a G-to-A transition in exon 4 of the *COMT* gene results in a heat-labile enzyme that is 4- to 5-fold less effective at methylating catechol substrates *in vitro* (50). The Met allele has been reported to result in 2- to 3-fold lower levels of methoxyestrogen metabolite formation in one (51), but not another (52), study. Both *GSTM1* and *GSTT1* have a deletion polymorphism (''null'' allele), which results in a complete lack of enzymatic activity (53, 54).

We investigated whether the polymorphisms in *CYP1A1*, *CYP1A2*, *CYP1B1*, *COMT*, *GSTM1*, and *GSTT1* described above, alone and in combination, affect endometrial cancer risk. Table 1 summarizes the polymorphisms, their possible functional significance based on laboratory studies, and their potential effect on endometrial cancer risk under the hypothesis that increased exposure to 2-OH estrogen might decrease, and increased exposure to 4-OH estrogen might increase, endometrial cancer risk.

Materials and Methods

Study Subjects. Participants from a population-based case-control study of endometrial cancer as well as a subset of controls from a population-based breast cancer case-control study [the Women's Contraceptive and Reproductive Experiences Study (CARE) Study (55)] conducted during the same period are included in this study. Because our endometrial cancer case-control study relied on the use of control women from the CARE study, which specifically studied only Caucasian and African American women, only Caucasian and African American women were recruited for the endometrial cancer study. Eligible case participants included Caucasian and African American female residents of western Washington state ages 50 to 69 years diagnosed with invasive endometrial cancer between January 1, 1994 and December 31, 1995 (King county only) and between July 1, 1997 and December 31, 1999 (King, Pierce, and Snohomish counties). These women were identified through the Cancer Surveillance System, a population-based tumor registry affiliated with the Surveillance, Epidemiology and End Results Program of the National Cancer Institute (56). Of the 582 eligible cases identified, 472 (81.1%) were successfully interviewed. Blood samples were provided by 383 (65.8%) of the eligible women (81.1% of interviewed women).

Eligible control women included Caucasian and African American female residents of the three-county area during the years the cases were diagnosed, with intact uteri and no prior history of endometrial cancer. They were selected from two sources [random-digit dialing (ref. 57; women ages 50-65 years) and random selection from Health Care Financing Administration data files (women ages 66-69 years)] and were frequency matched to the cases by 5-year age group

Table 1. Summary of polymorphisms in *CYP1A1*, *CYP1A2*, *CYP1B1*, *COMT*, *GSTM1*, and *GSTT1* examined in the current study and the expected direction of effect on risk of the presence of the variant allele under the hypothesis that an increased 2:4-hydroxylation ratio decreases risk of endometrial cancer

Gene	Primary pathway	Allele	Nucleotide change	Possible effect of the variant allele	Expected direction of effect on risk	References*
CYP1A1	2-Hydroxylation	''m1,'' *Msp*I	T6235C in 3′ noncoding region	Increased enzyme activity and inducibility	Decrease	(24–28)
		''m2''	Ile^{462}Val in exon 7	Increased enzyme activity and inducibility	Decrease	(27, 28, 32–34)
		''m4''	Thr^{461}Asn in exon 7	Decreased activity	Increase	(36, 38)
CYP1A2	2-Hydroxylation	C Val	A734C in intron 1	Decreased inducibility	Increase	(39, 40)
CYP1B1	4-Hydroxylation		Leu^{432}Val in exon 3	Higher 4:2-OH estradiol ratio	Increase	(42–48)
COMT	Converts catechol estrogens to inactive metabolites	Met	Val^{158}Met in exon 4	Decreased activity	Increase	(50, 51)
GSTM1	Phase II detoxification	''null''	Gene deletion	No activity	Increase	(53)
GSTT1	Phase II detoxification	''null''	Gene deletion	No activity	Increase	(54)

*Not all studies observed functional consequences of the polymorphisms studied, including refs. 29–31 for *CYP1A1* m1, refs. 26, 35–37 for *CYP1A1* m2, ref. 41 for *CYP1A2*, ref. 49 for *CYP1B1*, and ref. 52 for *COMT*.

and county of residence. The random-digit dialing screening response was 91.3%; we imputed that 18.2% of the never-answered numbers were residential and were therefore included in the denominator. Of the identified women, 83.6% were willing to be interviewed. The overall random-digit dialing response (the screening response multiplied by the interview response) was 76.3%, with 297 random-digit dialing controls interviewed. Of the 175 eligible Health Care Financing Administration controls, 116 (66.3%) agreed to an interview.

The CARE breast cancer study was conducted during the same period as the endometrial cancer case-control study using a similar questionnaire. Eligible population-based controls from this study were included in the endometrial cancer study. The CARE study controls included Caucasian and African American women ages 35 to 64 years ascertained through random-digit dialing in five metropolitan areas of the United States, including King County, WA, between 1994 and 1998. The overall levels of screening and interview response for King County were 83.6% and 88.3%, respectively. We invited 132 King County CARE control women ages 50 to 64 years, with intact uteri, to provide a blood sample, and we successfully obtained a blood sample from 115. Overall, of the 930 eligible controls, 665 (71.5%) were interviewed and 450 (48.4%) provided a blood sample (67.7% of interviewed controls).

The data from one control in the earlier case-control study, who was ascertained as a case in the later case-control study, was included in both case and control groups; one case was excluded because of poor quality interview data; and four controls provided blood after the genotyping for this study had ended. Additionally, there were only 11 cases and 26 controls who were Hispanic or non-Caucasian, so we were unable to stratify our analyses on racial subgroups. To reduce the possibility of observing spurious results due to population stratification, we restricted our analyses to non-Hispanic Caucasian women, leaving us with a total of 371 cases and 420 controls.

After informed consent, all participants were administered an in-person interview conducted according to a standard protocol. Each participant was asked only about events that occurred before her reference date, which is the date of diagnosis for cases. Controls were assigned a reference date based on the distribution of diagnosis years for the cases. Data were collected on demographic factors; height; weight at different ages; reproductive, contraceptive, and menstrual history; family history of cancer; history of selected chronic conditions; and history of contraceptive and noncontraceptive hormone use. Color pictures of oral contraceptive and hormone replacement therapy pill packs were used to aid recall. Interviews for the endometrial cancer case-control study and the CARE controls were essentially the same. The protocols of both studies were approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center (Seattle, WA).

Genotype Data. Consenting participants provided venous blood samples from which DNA was extracted using a salting-out procedure (58). We used PCR-RFLP methods for genotyping. We included positive controls with known genotypes and negative controls (reaction mixtures without DNA templates) in each run of our genotype assays. We conducted our pre-PCR work and post-PCR work in separate rooms and used pipette tips fitted with filters to avoid contamination from aerosol. The laboratory staff were blinded to patient characteristics.

All PCR assays were done with the following conditions: a 20 μL reaction contained 1× PCR buffer (Qiagen, Valencia, CA), 1.5 mmol/L $MgCl_2$, 0.5 units Taq DNA polymerase (Qiagen, Valencia, CA), 200 μmol/L deoxynucleotide triphosphates (Roche Diagnostics, Indianapolis, IN), 100 ng DNA, and 100 nmol/L of each primer except for *GSTT1* in which 200 nmol/L of each primer was used. The PCR products were digested with 5 units of restriction enzymes per manufacturer's instructions (New England Biolabs, Beverly, MA), separated on an agarose gel, and visualized by UV after staining with ethidium bromide. The *CYP1A1* m2 and m4 polymorphisms are 2 bp apart and both affect the recognition sequence of the restriction enzyme *Nco*I used in the triplex assay for the *GSTT1*, *GSTM1*, and *CYP1A1* m2 polymorphisms outlined by Bailey et al. (59). We modified the assay to definitively distinguish between *CYP1A1* m2 and m4 polymorphisms using primers 5′-GAAAGG-CTGGGTCCACCCTCT-3′ and 5′-CCAGGAAGAGAAA-GACCTCCCAGCGGTC-3′. The second primer creates a *Hin*cII restriction enzyme site that cuts when the *CYP1A1* m2 allele is G but does not cut when it is an A. The recognition sequence is not affected by *CYP1A1* m4 status. *CYP1A1* m2 A allele homozygotes are represented as 182- and 151-bp bands on agarose gel, whereas G homozygotes are represented as 182-, 120-, and 31-bp bands; heterozygotes show all bands. Table 2 contains the primer sequences, thermocycling conditions, restriction enzymes, and gel variables for each assay.

Statistical Analyses. For each polymorphism, deviation from Hardy-Weinberg equilibrium was tested using the standard Pearson χ^2 test (Stata version 8.2 ''genhwcci'' command). *CYP1A1*, *CYP1A2*, and *CYP1B1* alleles were defined as low-risk or high-risk based on the results of published functional studies and the hypothesized effect that the variant would have on the ratio of 2- to 4-hydroxylation, with the highest ratio of 2- to 4-hydroxylation considered to represent the lowest risk for endometrial cancer (see Table 1). Odds ratios (OR) and 95% confidence intervals (95% CI) for each putative low-risk or high-risk genotype and endometrial cancer were calculated using unconditional logistic regression. Homozygotes and heterozygotes for each of the variant alleles were compared with homozygotes for the wild-type allele. If homozygous variant genotypes occurred too infrequently to allow them to be assessed separately, they were combined with heterozygotes to increase statistical efficiency. For *GSTM1* and *GSTT1*, individuals lacking both copies of the gene (null) are compared with carriers of at least one copy of the gene (present).

We estimated *CYP1A1* haplotype frequencies and calculated ORs and 95% CIs to estimate their associations with endometrial cancer risk, using the most common haplotype as the reference category, with the Hplus program (http://cougar.fhcrc.org/hplus; ref. 60). This program estimates the probability of carriage of each of the possible haplotypes for each individual and uses this distribution in the estimation of risk. Haplotypes are treated as unobserved latent variables, and estimating equations are constructed by integrating out these latent haplotypes. The haplotype analysis specifies a logistic penetrance function relating haplotypes and covariates with the disease outcome. The coefficients are estimated through generalized estimating equations, integrated over all possible phases for the latent haplotypes via the conditional expectation of the estimating function given the data. One strength of this program is that it incorporates the error generated from estimating haplotypes into the OR and 95% CI estimates. The program has been validated using simulations (60, 61).

We also combined genotypes, including all the polymorphisms in the genes involved in the 2- and 4-hydroxylation pathways for which we had data (i.e., *CYP1A1*, *CYP1A2*, and *CYP1B1*), to determine whether certain combinations of the putative low-risk and high-risk genotypes were associated with endometrial cancer risk. Each of the polymorphisms was classified as carriage of one or more variant alleles versus none. The genotype combinations were assigned a value that represented the sum of the

Table 2. Primer sequences, thermocycling conditions, restriction enzymes, and gel variables used to genotype variants in *CYP1A1*, *CYP1A2*, *CYP1B1*, *COMT*, *GSTM1*, and *GSTT1*

Polymorphism	Primer sequences	Thermocycling conditions	Restriction enzyme (5 units); gel variables	References
CYP1A1 m1	5′-GGCTGAGCAATCTGACCCTA-3′, 5′-GGCCCCAACTACTCAGAGGCT-3′	95°C, 2 min; 35 × 94°C, 1 min; 58°C, 30 s; 72°C, 1.5 min 72°C, 5 min;	*Msp*I, *Sph*I; 4% NuSieve GTG (FMC, Rockland, ME)	(59)
(Triplex PCR) *CYP1A1* m2 or m4	5′-GAAAGGCTGGGTCCACCCTCT-3′, 5′-CCAGGAAGAGAAAGACCTCCCAGCGGGCCA-3′	95°C, 2 min; 35 × 94°C, 1 min;	*Nco*I, *Hin*fI; 2.5% agarose (Invitrogen, Carlsbad, CA)	(59)
GSTM1 (deletion)	5′-CTGCCCTACTTGATTGATGGG-3′, 5′-CTGGATTGTAGCAGATCATGC-3′	60°C, 1 min; 72°C, 2 min; 72°C, 5 min		
GSTT1 (deletion)	5′-TTCCTTACTGGTCCTCACATCTC-3′, 5′-TCACCGGATCATGGCCAGCA-3′			
CYP1A1 m2	5′-GAAAGGCTGGGTCCACCCTCT-3′, 5′-CCAGGAAGAGAAAGACCTCCCAGCGGTC-3′	95°C, 2 min; 35 × 94°C, 1 min; 60°C, 1 min; 72°C, 2 min; 72°C, 5 min	*Hin*cII; 4% NuSieve GTG (FMC, Rockland, ME)	This study
CYP1A1 m4	5′-GAAAGGCTGGGTCCACCCTCT-3′, 5′-GGCCCCAACTACTCAGAGGCT-3′	95°C, 2 min; 35 × 94°C, 1 min; 62°C, 30 s; 72°C, 1.5 min; 72°C, 5 min	*Bsa*I; 2.5% agarose (Invitrogen, Carlsbad, CA)	(59)
CYP1A2	5′-CAACCCTGCCAATCTCAAGCAC-3′, 5′-AGAAGCTCTGTGGCCGAGAAGG-3′	95°C, 2 min; 40 × 94°C, 1 min; 62°C, 30 s; 72°C, 1 min; 72°C, 5 min	*Apa*I; 2.5% agarose (Invitrogen, Carlsbad, CA)	(40)
CYP1B1	5′-TAAGAATTTTGCTCACTTGC-3′, 5′-GTTCTCCGGGTTAGGCCACTTAA-3′	95°C, 2 min; 15 × 94°C, 1 min; 60°C, 30 s; 72°C, 1 min; 25 × 94°C, 1 min; 50°C, 30 s; 72°C, 1 min; 72°C, 5 min	*Afl*II; 4% NuSieve GTG (FMC, Rockland, ME)	(76)
COMT Val^{158}Met	5′-TACTGTGGCTACTCAGCTGTGC-3′, 5′-GTGAACGTGGTGTGAACACC-3′	95°C, 2 min; 40 × 94°C, 1 min; 62°C, 1 min; 72°C, 2 min; 72°C, 5 min	*Nla*III; 4% NuSieve GTG (FMC, Rockland, ME)	(77)

total number of presumed low-risk genotypes (according to Table 1).

To explore whether the possible effect of decreased *O*-methylation varies by the relative amounts of 2- and 4-OH estrogen produced, *COMT* genotypes were examined separately by each level of the combined *CYP1A1*, *CYP1A2*, and *CYP1B1* genotype classification. In addition, we constructed a logistic regression model with a multiplicative term for *COMT* and the combined genotype. The *P* was computed for the likelihood ratio test, comparing logistic regression models with and without the multiplicative term. Similar analyses were done to examine any possible modifying effects of the combined CYP gene variable and *GSTM1* and *GSTT1* null genotypes.

We adjusted our analyses for age. We did not include other characteristics (e.g., reference year, body mass index, hormone replacement therapy use, oral contraceptive use, cigarette smoking, and parity) in our analyses because they did not alter our results for the stratum-specific estimates or those for the combined genotypes by >10%. All tests of statistical significance were two sided.

Results

Characteristics of cases and controls are presented in Table 3. Women with endometrial cancer were more likely to have had a high body mass index, to have used unopposed estrogens, and never to have given birth. They were less likely to have used oral contraceptives or smoke cigarettes (Table 3).

Among the controls, genotype frequency distributions for the polymorphisms studied did not deviate to any appreciable extent from expectation predicted by the Hardy-Weinberg equilibrium (Table 4). Carrying at least one *CYP1A1* m1 C allele was associated with a decreased risk of endometrial cancer (OR, 0.64; 95% CI, 0.44-0.93), as was carrying at least one *CYP1A1* m2 Val allele (OR, 0.54; 95% CI, 0.30-0.99; Table 4). The homozygous variant genotype was rare for both m1 and m2 variants (present in 2% and <1% of controls, respectively). The presence of the *CYP1A1* m4 allele was not associated with endometrial cancer risk (OR, 1.10; 95% CI, 0.67-1.80; Table 4), but we were unable to assess whether carriage of the homozygous variant genotype confers risk of endometrial

cancer because only one case, and no controls, had that genotype. The results from the estimated *CYP1A1* haplotypes were nearly identical to those obtained from examining each genotype separately. Carriage of the *CYP1A1* m1 C allele was associated with a decreased risk of endometrial cancer without the presence of the m2 and m4 variant alleles, and the risk was further decreased when the m1 C and m2 Val alleles were carried in combination (Table 5).

There was at most a weak elevation in risk associated with the *CYP1A2* CC genotype compared with the AA genotype (OR, 1.24; 95% CI, 0.72-2.12) as well as the *CYP1B1* Val/Val genotype compared with the Leu/Leu genotype (OR, 1.34; 95% CI, 0.90-1.98; Table 4). Carriage of just one of the *CYP1A2* C alleles or of the *CYP1B1* Val alleles was not associated with endometrial cancer risk. Contrary to our expectations, the *COMT* Met/Val and Met/Met genotypes were associated with a weakly decreased risk of endometrial cancer compared with the Val/Val genotype [ORs and 95% CIs, 0.78 (0.55-1.10) and 0.75 (0.51-1.11)]. The *GSTT1* null genotype was associated with an increased risk of endometrial cancer (OR, 1.55 95% CI, 1.07-2.24), although the *GSTM1* null genotype was not (Table 4).

Table 3. Characteristics of endometrial cancer cases and controls

Characteristic	Cases* (n = 371), n (%)	Controls* (n = 420), n (%)
Age (y)		
50-54	94 (25)	130 (31)
55-59	103 (28)	103 (25)
60-64	97 (26)	101 (24)
65-69	77 (21)	86 (20)
Education (y)		
≤12	35 (9)	29 (7)
13-16	219 (59)	217 (52)
≥17	117 (32)	174 (41)
No. births		
0	65 (18)	43 (10)
1	46 (12)	35 (8)
≥2	260 (70)	342 (81)
Oral contraceptive use (y)†		
Never	143 (39)	127 (31)
<5	150 (41)	157 (39)
≥5	76 (21)	122 (30)
Cigarette smoking		
Never	201 (54)	178 (42)
Former (>1 y ago)	132 (36)	169 (40)
Current (in the past year)	38 (10)	73 (17)
Body mass index (kg/m^2)‡		
<21.97	62 (17)	104 (25)
21.97-24.21	47 (13)	104 (25)
24.22-28.29	101 (27)	107 (25)
≥28.29	160 (43)	105 (25)
Postmenopausal hormone regimen use§		
Never	153 (42)	182 (44)
Only unopposed estrogen	37 (10)	13 (3)
Only estrogen + progestin	121 (33)	176 (42)
Unopposed estrogen and estrogen + progestin	46 (13)	41 (10)
Unopposed progestin and estrogen + progestin	4 (1)	1 (<1)
Only unopposed progestin	6 (2)	3 (1)

*Distributions of characteristics are reported for non-Hispanic Caucasian women only, because there were too few African American and/or Hispanic women (11 cases and 26 controls) to either stratify on or control for race/ethnicity. Therefore, they were excluded from analyses.

†Two cases and 14 controls either used sequential oral contraceptives or used unknown oral contraceptives during that time when sequential oral contraceptives were marketed; these data are not included above.

‡Data were missing for body mass index for one case.

§Postmenopausal use of estrogen and progestin hormonal pills or patches for ≥6 months. Data were missing for four cases and four controls.

The combined genotypes using all of the polymorphisms we assessed in genes involved in estrogen hydroxylation (*CYP1A1*, *CYP1A2*, and *CYP1B1*), ranked by the number of putative low-risk genotypes carried (according to Table 1), are shown in Table 6. The first column lists the *number* of putative low-risk genotypes carried, and the ''putative low-risk genotypes'' columns specify which of the putative low-risk genotypes in *CYP1A1*, *CYP1A2*, and *CYP1B1* were carried (denoted by X in the relevant column). ORs and 95% CIs were calculated using carriage of one or none of the putative low-risk genotypes as the reference group. Carriage of four or five of the putative low-risk genotypes was associated with a reduced risk of endometrial cancer (OR, 0.29; 95% CI, 0.15-0.56), and there was no appreciable alteration in risk among women carrying two or three of the putative low-risk genotypes (Table 6).

We attempted to explore whether our data would provide evidence that 2-OH estrogens inhibit the *O*-methylation of 4-OH estrogens (and possibly allow 4-OH estrogens to accumulate) by examining the risk associated with carriage of the low activity *COMT* allele by the categories of the combined *CYP1A1*, *CYP1A2*, and *CYP1B1* genotypes. If this were true, we would have expected to observe an increased risk of endometrial cancer associated with carrying the low-activity *COMT* Met allele (compared with the Val allele) among women carrying the combined *CYP1A1*, *CYP1A2*, and *CYP1B1* genotypes that may result in the highest relative 2-OH estrogen levels. Alternatively, it might be expected that the *COMT* low-activity allele would be associated with an increased risk in the subgroup of women who might have produced the highest relative levels of 4-OH estrogen. Contrary to what we expected, the *COMT* low-activity allele was not associated with an increased risk of endometrial cancer in any of the subgroups; indeed, there was a suggestion of a decreased risk among women carrying genotype combinations that are thought to result in the lowest 2:4-hydroxylation ratio (carriage of one and none of the low-risk alleles; OR, 0.58; 95% CI, 0.32-1.04; Table 7). There was little evidence of any interaction between the combined *CYP1A1*, *CYP1A2*, and *CYP1B1* genotypes and *GSTM1* or *GSTT1* genotypes (Table 7), although the study has limited statistical power to detect such an interaction.

Discussion

Our study sought to examine whether variants in genes involved in catechol estrogen formation and metabolism are associated with endometrial cancer risk. Although we examined the association between eight individual polymorphisms and endometrial cancer risk, we chose not to control for multiple testing because of our strong *a priori* hypotheses, which were based on biological evidence of the functional significance of the genotypes. We based the interpretation of our results on the magnitude and direction of the ORs, not statistical significance. We hypothesized that endometrial cancer incidence would be relatively lower in women with genotypes that might be associated with inferred higher estrogen 2-hydroxylation and lower 4-hydroxylation and higher among women with an inferred impaired ability to convert the putative high-risk 4-hydroxylated metabolites to less harmful compounds and the 2-hydroxylated metabolites to compounds thought to be protective (see Table 1). Specifically, based on experimental evidence indicating that the *CYP1A1* m1 and m2 variants might be associated with higher enzyme activity and/or inducibility (24-28) and therefore possibly increased 2-OH estrogen formation, we hypothesized that they might be associated with a decreased

Table 4. *CYP1A1*, *CYP1A2*, *CYP1B1*, *COMT*, *GSTM1*, and *GSTT1* genotypes and endometrial cancer risk

Genotype	Cases (n = 371), n (%)	Controls (n = 420), n (%)	OR, age adjusted (95% CI)	P for HWE in controls
CYP1A1 m1				
TT	317 (85)	331 (79)	1.00 (Reference)	0.12
≥1 C	54 (15)	89 (21)	0.64 (0.44-0.93)	
TC	53 (14)	80 (19)	0.69 (0.47-1.02)	
CC	1 (<1)	9 (2)	0.12 (0.02-0.95)	
CYP1A1 m2				
Ile/Ile	354 (95)	386 (92)	1.00 (Reference)	0.74
≥1 Val	17 (5)	34 (8)	0.54 (0.30-0.99)	
Ile/Val	17 (5)	33 (8)	—	
Val/Val	0 (0)	1 (<1)	—	
CYP1A1 m4				
Thr/Thr	337 (91)	384 (91)	1.00 (Reference)	0.36
≥1 Asn	34 (9)	36 (9)	1.10 (0.67-1.80)	
Thr/Asn	33 (9)	36 (9)	—	
Asn/Asn	1 (<1)	0 (0)	—	
CYP1A2				
AA	197 (53)	221 (53)	1.00 (Reference)	0.63
≥1 C	174 (47)	199 (47)	0.98 (0.74-1.30)	
AC	142 (38)	170 (40)	0.93 (0.69-1.25)	
CC	32 (9)	29 (7)	1.24 (0.72-2.12)	
CYP1B1				
Leu/Leu	115 (31)	145 (35)	1.00 (Reference)	0.27
≥1 Val	256 (69)	275 (65)	1.18 (0.87-1.59)	
Val/Leu	170 (46)	194 (46)	1.11 (0.81-1.53)	
Val/Val	86 (23)	81 (19)	1.34 (0.90-1.98)	
COMT				
Val/Val	97 (26)	90 (21)	1.00 (Reference)	0.87
≥1 Met	274 (73)	330 (78)	0.77 (0.55-1.07)	
Val/Met	174 (47)	207 (49)	0.78 (0.55-1.10)	
Met/Met	100 (27)	123 (29)	0.75 (0.51-1.11)	
GSTM1				
Present	190 (51)	200 (48)	1.00 (Reference)	NA*
Null	181 (49)	220 (52)	0.87 (0.65-1.15)	
GSTT1				
Present	293 (79)	359 (85)	1.00 (Reference)	NA*
Null	78 (21)	61 (15)	1.55 (1.07-2.24)	

*HWE could not be tested because the assay detects the presence or absence of the allele, not the number of copies.

risk of endometrial cancer. We did indeed observe a decreased risk associated with carriage of the *CYP1A1* m1 C and m2 Val alleles [ORs and 95% CIs, 0.64 (0.44-0.93) and 0.54 (0.30-0.99)]. Because the *CYP1A1* m4 Asn allele has been reported to be associated with decreased activity (36, 38), we expected it to be associated with an increased risk, but it was not.

Consistent with our results, in a small study (reported in abstract form; 43 cases and 36 controls), possessing at least one *CYP1A1* m2 allele was associated with a decreased risk of endometrial cancer (OR, 0.51; 95% CI, 0.13-1.97; ref. 62). However, in a Spanish hospital-based study of endometrial cancer (80 cases and 60 controls), ORs and 95% CIs associated with the presence of the *CYP1A1* m1, m2, and m4 variant alleles were 3.67 (1.21-13.26), 3.67 (1.21-13.26), and 6.36 (1.99-26.5), respectively (63, 64). ORs (95% CIs) of 0.88 (0.33-2.35) and 1.7 (0.63-4.57) for carriers of the m1 and m2 variant alleles, respectively, were observed in a small Japanese study (38 cases and 31 controls; ref. 65).

We observed a modest increased risk associated with the *CYP1B1* Val/Val genotype. Two other studies have examined the association between this variant and endometrial cancer risk. Although there was no association reported in a nested endometrial cancer case-control study within the Nurses' Health study cohort (222 cases and 666 controls; ref. 66), a Japanese study (113 cases and 202 controls) observed that carriers of the Val/Val genotype were at a 2.5-fold increased risk of endometrial cancer (95% CI, 1.10-5.66; ref. 67). These two studies also reported results for other polymorphisms in the *CYP1B1* gene, with inconsistent results.

When alleles from *CYP1A1*, *CYP1A2*, and *CYP1B1* were combined into genotypes that we hypothesized might be associated with high versus low 2- to 4-hydroxylation ratios and ranked by the number of low-risk genotypes carried, we observed a 71% decreased risk associated with carriage of four and five low-risk genotypes compared with carriage of no more than one. These results suggest that a relative increase in 2-OH estrogen (and a decrease in 4-OH estrogen) might be associated with a decreased risk of endometrial cancer.

We expected that the *COMT* low-activity allele would be associated with an increased risk of endometrial cancer,

Table 5. *CYP1A1* haplotypes and endometrial cancer risk

Polymorphism			Cases (n = 371)		Controls (n = 420)		OR, age adjusted (95% CI)
m1	m2	m4	n	Frequency	n	Frequency	
T	Ile	Thr	326	0.880	353	0.841	1.00 (Reference)
T	Ile	Asn	17	0.046	18	0.043	1.10 (0.67-1.78)
C	Ile	Thr	19	0.050	32	0.075	0.67 (0.45-1.01)
C	Val	Thr	9	0.023	17	0.041	0.54 (0.30-0.97)

Table 6. ORs and 95% CIs for endometrial cancer risk in relation to the number of putative low-risk *CYP1A1*, *CYP1A2*, and *CYP1B1* genotypes carried

No. putative low-risk genotypes carried	Cases (n = 371)		Controls (n = 420)			Putative low-risk genotypes*				
	n	Frequency	n	Frequency	OR, age adjusted (95% CI)	*CYP1A1* m1 ≥1 C	*CYP1A1* m2 ≥1 Val	*CYP1A1* m4 Thr/Thr	*CYP1A2* AA	*CYP1B1* Leu/Leu
0	9	0.024	5	0.012		—	—	—	—	—
1	102	0.275	111	0.264		—	—	X	—	—
1	15	0.040	15	0.036		—	—	—	X	—
1	3	0.008	3	0.007		—	—	—	—	X
0 and any 1	**129**	**0.347**	**134**	**0.319**	**1.00 (Reference)**					
2	4	0.011	10	0.024		X	—	X	—	—
2	1	0.003	2	0.004		X	—	—	X	—
2	89	0.240	93	0.221		—	—	X	X	—
2	44	0.119	54	0.129		—	—	X	—	X
2	4	0.011	9	0.021		—	—	—	X	X
Any 2	**142**	**0.384**	**168**	**0.399**	**0.89 (0.64-1.24)**					
3	11	0.030	6	0.014		X	X	X	—	—
3	0	0	1	0.002		X	X	—	X	—
3	22	0.059	19	0.045		X	—	X	X	—
3	1	0.003	4	0.010		X	—	X	—	X
3	2	0.005	0	0		X	—	—	X	X
3	51	0.137	41	0.098		—	—	X	X	X
Any 3	**87**	**0.234**	**71**	**0.169**	**1.27 (0.85-1.89)**					
4	3	0.008	13	0.031		X	X	X	X	—
4	0	0	6	0.014		X	X	X	—	X
4	0	0	1	0.002		X	X	—	X	X
4	7	0.019	20	0.048		X	—	X	X	X
5	3	0.008	7	0.017		X	X	X	X	X
Any 4 and 5	**13**	**0.035**	**47**	**0.112**	**0.29 (0.15-0.56)**					

NOTE: For example, in the first row, with a ''0'' in column 1, 9 (2.4%) cases and 5 (1.2%) controls carried a genotype combination that included no low-risk genotypes at *CYP1A1* m1, m2, and m4; *CYP1A2*; and *CYP1B1* (designated by a ''—'' in each of those columns). In the second to last row, with a ''5'' in column 1, 3 (0.8%) cases and 7 (1.7%) controls carried a low-risk genotype at all loci (designated by a ''X'' in each of the columns). In the last row, with ''Any 4 and 5'' in column 1, the numbers and frequencies in bold are the sums of all the rows containing genotypes with four and five putative low-risk genotypes.
*''X'' in the column indicates the presence of the genotype. Only genotype combinations that were present in the cases or controls are shown.

particularly among women who had either an inferred high levels of 4-OH estrogens or an inferred high ratio of 2- to 4-OH estrogens (because high levels of 2-OH estrogens can inhibit the *O*-methylation of 4-OH estrogens; ref. 11), but our results did not support this hypothesis. In our study, the Met allele was associated with a modest *decreased* risk (OR, 0.77; 95% CI, 0.55-1.07). When we examined the *COMT* genotypes by number of putative low-risk genotypes carried in *CYP1A1*,

Table 7. Risk of endometrial cancer associated with *COMT*, *GSTM1*, and *GSTT1* genotypes, stratified by number of putative low-risk genotypes in *CYP1A1*, *CYP1A2*, and *CYP1B1*

No. putative low-risk genotypes in *CYP1A1*, *CYP1A2*, and *CYP1B1*	Gene	Cases (n = 371), n (%)	Controls (n = 420), n (%)	OR, age adjusted (95% CI)
	COMT			
0 and 1	Val/Val	37 (10)	25 (6)	1.00 (Reference)
	≥1 Met	92 (25)	109 (26)	0.58 (0.32-1.04)
2 and 3	Val/Val	57 (15)	58 (14)	1.00 (Reference)
	≥1 Met	172 (46)	181 (43)	0.96 (0.63-1.46)
4 and 5	Val/Val	3 (1)	7 (2)	1.00 (Reference)
	≥1 Met	10 (3)	40 (10)	0.92 (0.17-4.83)
				$P_{interaction}$ = 0.33
	GSTM1			
0 and 1	Present	67 (18)	64 (15)	1.00 (Reference)
	Null	62 (17)	70 (17)	0.79 (0.48-1.30)
2 and 3	Present	116 (31)	115 (27)	1.00 (Reference)
	Null	113 (30)	124 (30)	0.91 (0.63-1.30)
4 and 5	Present	7 (2)	21 (5)	1.00 (Reference)
	Null	6 (2)	26 (6)	0.44 (0.11-1.83)
				$P_{interaction}$ = 0.91
	GSTT1			
0 and 1	Present	107 (29)	115 (27)	1.00 (Reference)
	Null	22 (6)	19 (5)	1.29 (0.65-2.56)
2 and 3	Present	177 (48)	203 (48)	1.00 (Reference)
	Null	52 (14)	36 (9)	1.66 (1.04-2.66)
4 and 5	Present	9 (2)	41 (10)	1.00 (Reference)
	Null	4 (1)	6 (1)	2.62 (0.54-12.61)
				$P_{interaction}$ = 0.54

CYP1A2, and *CYP1B1*, there was a suggestion that the *COMT* Met allele was associated with a *decreased* risk just in the group carrying no more than one low-risk genotype (which is possibly the group with the highest level of 4-OH estrogen and the lowest level of 2-OH estrogen). Our study has limited power to detect such an interaction, and these results are presented only for the purposes of generating hypotheses.

The other study to date to examine the association between the *COMT* Met allele and endometrial cancer risk reported an OR close to 1 (66). It could be that even a low-activity form of the COMT enzyme might be capable of converting enough of the 2-OH estrogen into 2-methoxyestrogen for it to exert a protective or neutral role with respect to endometrial cancer risk. Alternatively, it has been reported that estradiol can reduce *COMT* expression through an estrogen receptor–mediated mechanism (68). Because the majority of women who develop endometrial cancer have relatively high estradiol levels, this possible reduction in *COMT* expression may outweigh the effect that the Val^{158}Met polymorphism has on the relative production of 2- and 4-hydroxylated metabolites. Furthermore, although one study reported decreased methoxyestrogen formation associated with the Met allele in an *Escherichia coli* expression system and in human breast cancer cell lines (51), another study failed to find such a difference (52). Finally, the pathways considered in this article may prove to be more complex; in addition to the roles *CYP1A1* and *CYP1B1* play in the production of catechol estrogens via estrogen hydroxylation, it has been observed that these enzymes can demethylate methoxyestrogens back into catechol estrogens and that methoxyestrogens decrease the production of catechol estrogens by feedback inhibition on *CYP1A1* and *CYP1B1* (69). Given that *COMT* is expressed in the endometrium, it is a promising candidate gene to examine in relation to endometrial cancer risk. It is possible that other polymorphisms in this gene may prove to be more relevant than the one we investigated.

In the study by Esteller et al. (63), the *GSTM1*, but not the *GSTT1*, null genotype was associated with an increased risk of endometrial cancer (OR, 2.01; 95% CI, 0.9-4.2). However, in the current study, only the *GSTT1* null genotype was associated with an increased risk (OR, 1.55; 95% CI, 1.07-2.24). Even if one or both of these *GST* genotypes were truly associated with the risk of endometrial cancer, it is not entirely clear what the mechanistic role of the GSTM1 and GSTT1 enzymes might be. GSTs are probably involved in the deactivation of estrogen-derived quinones (21), but it is not clear which of the GSTs are involved. A recent report showed that GSTP1 has this capability, and the authors suggest that because the GSTs have overlapping substrate specificity it is likely that other GSTs share this property (70). In addition, GSTM1 has been observed to deactivate equine catechol estrogen quinones through reduced glutathione conjugation (71). The GSTs are also involved in catabolism of some environmental carcinogens (such as polycyclic aromatic hydrocarbons in cigarette smoke) that are activated by CYP1A1, CYP1A2, and CYP1B1 (72). It is possible that an association with polymorphisms in the *GST* genes as well as the *CYP1A1*, *CYP1A2* and *CYP1B1* genes could be due to the role that they play in another metabolic pathway with substrates other than estrogen and its metabolites.

There is no obvious explanation for the inconsistent results observed in studies of the genotypes above and endometrial cancer risk. Given that most of the risk estimates reported to date are based on a few study participants and that the expected true effect of the allele (if any) would be small, sampling variability and low study power is perhaps the most plausible explanation for many of the between-study differences in risk estimates. Another possibility is a combination of (*a*) a true difference in the relative risk associated with the presence of a particular allele according to the presence or absence of another etiologic factor (e.g., unopposed estrogen use or obesity) and (*b*) a difference in the prevalence of that other factor in the various populations in which the studies had been conducted. Confounding by race and selection bias may also be issues. Unfortunately, from just the data provided in published reports of these studies, these potential explanations generally cannot be evaluated.

Although our results from the combined *CYP1A1*, *CYP1A2*, and *CYP1B1* genotypes are consistent with the hypothesis that a relative increase in estrogen 2-hydroxylation compared with 4-hydroxylation might be associated with a reduced risk of endometrial cancer, our results for *COMT* do not necessarily provide support for this hypothesis. Our results would be strengthened by additional genotype information, particularly for other common, possibly functional polymorphisms in the genes studied that have been described and characterized since our genotyping for this research began. Other genes of potential interest are *CYP3A4* and *CYP3A5*, involved in hepatic estrogen 2-hydroxylation (73); NAD(P)H:quinone oxidoreductase, involved in preventing catechol estrogen from redox cycling (74); and manganese superoxide dismutase, involved in reducing oxidative stress caused by redox cycling (75). Building multigenic, pathway-based models of endometrial cancer risk could well aid in our understanding of this disease and our understanding of the actions of estrogens and their precursors and metabolites.

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