

Estrogen Sulfation Genes, Hormone Replacement Therapy, and Endometrial Cancer Risk

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Background: Unopposed estrogen replacement therapy is associated with increased risk of endometrial cancer. To investigate the mechanism of this association, we evaluated whether risk of endometrial cancer was associated with the genotypes involved in steroid hormone metabolism and the duration of exogenous hormone use. **Methods:** A population-based case-control study in nine counties of the Philadelphia metropolitan area was undertaken with 502 case patients with endometrial cancer and 1326 age- and race-matched control subjects. Data regarding exogenous hormone use were obtained by interview, and genotypes of the genes COMT, CYP1A1, CYP1A2, CYP1B1, CYP3A4, PGR, SULT1A1, SULT1E1, and UGT1A1 were obtained by polymerase chain reaction techniques. Conditional logistic regression was used to examine the relationship among genotype, hormone use, and endometrial cancer risk. **Results:** Associations were observed between the risk of endometrial cancer and genotypes of the following steroid hormone metabolism genes: CYP1A1*2C (adjusted odds ratio [OR] = 1.68, 95% confidence interval [CI] = 1.08 to 2.61); SULT1A1*3 (adjusted OR = 0.51, 95% CI = 0.29 to 0.92); and the G → A variant in the promoter of SULT1E1 at position -64 (adjusted OR = 1.45, 95% CI = 1.06 to 1.99). We observed a statistically significant interaction between estrogen replacement therapy use and SULT1A1*2 genotype: the SULT1A1*2 allele and long-term use of estrogen replacement therapy were associated with statistically significantly higher risk of endometrial cancer (adjusted OR = 3.85, 95% CI = 1.48 to 10.00) than that of the SULT1A1*2 allele and no estrogen replacement therapy use. **Conclusions:** Among women with long-term use of estrogen replacement therapy or combined hormone replacement therapy, the risk of endometrial cancer may be associated with functionally relevant genotypes that regulate steroid hormone sulfation. [J Natl Cancer Inst 2006;98:1311–20]

Postmenopausal hormone replacement therapy—including unopposed estrogen replacement therapy and combined hormone replacement therapy, involving combinations of estrogen and progestins—has been widely used to treat the consequences of menopause (1). However, there have also been serious concerns about adverse effects of exogenous hormone use, including increased risks of pulmonary embolism (2–5), stroke (6), coronary heart disease (7), and cancer (8–13). Although high rates of endometrial cancer are associated with the use of unopposed estrogen replacement therapy (8,14–17), the use of combined hormone replacement therapy is not associated with the same high risk of endometrial cancer (14,18). Many studies have found that combined hormone replacement therapy is associated with a

decreased risk of endometrial cancer (10–13,19–24). Different hormone exposure regimens and definitions, as well as different study designs and sample sizes, may account for differences in the inferences across these studies. In addition, it is possible that some populations or population subgroups may have different susceptibility to the effects of estrogen replacement therapy and/or combined hormone replacement therapy because of interindividual variation in the metabolism of these compounds.

From these observations, we hypothesized that genes involved in the metabolism of estrogen and/or progestins may modify the effects of estrogen replacement therapy and/or combined hormone replacement therapy that increase the risk of cancer. Candidate genes that are involved in hormone metabolism include CYP17 (25–27), CYP19 (26–28), HSD17β1 (29), CYP1B1 (30–33), CYP1A2 (32), CYP1A1 (32), COMT (30,32), GSTM1 (32), GSTT1 (32), estrogen receptor ESR2 (34), progesterone receptor PGR (35), and UGT1A1 (36). The results of these previous studies (25–36) support the hypothesis that these genes may be associated with the risk of endometrial cancer. However, studies to date have not evaluated whether these genes interact with exogenous hormone exposures to modify the risk of endometrial cancer. Therefore, we evaluated whether there was evidence for effect modification of estrogen replacement therapy and/or combined hormone replacement therapy exposure by genes involved in the metabolism of these compounds.

SUBJECTS AND METHODS

Study Design and Data Collection

The Women's Insights and Shared Experiences study is a population-based case-control study of case patients with incident

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See "Notes" following "References."

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endometrial cancer and frequency-matched control subjects selected from the community by random-digit dialing. Full details of our study design have been previously reported (14,37). The source population for this study was the contiguous nine-county region around Philadelphia (Bucks, Chester, Delaware, Montgomery, and Philadelphia Counties in Pennsylvania; Burlington, Camden, and Gloucester Counties in New Jersey; and New Castle County in Delaware). Potentially eligible case patients were African American or white women residing in these counties who were newly diagnosed with endometrial cancer between July 1, 1999, and June 30, 2002, and aged 50–79 years at the time of diagnosis. The case patients were identified through active surveillance at 61 hospitals that were open during this period. Quarterly reviews of the Pennsylvania Cancer Registry lists were used to validate the completeness of our case ascertainment. Pathology reports and medical records were reviewed by trained abstractors to validate diagnoses and to obtain information about tumor type, size, and grade; degree of metastasis; and lymph node involvement. Case status was validated by a pathology report that was compatible with primary, invasive, epithelial endometrial adenocarcinoma of all stages (I through IV) and all grades. Women were excluded if they had endometrial hyperplasia only, benign uterine lesions, lesions metastatic to the uterus, carcinoma in situ of the cervix, endolymphatic stromal lysis, mixed mesodermal mixed müllerian, leiomyosarcoma, carcinosarcoma, undifferentiated carcinoma, or squamous cell carcinoma. Case patients with clear cell and papillary serous cancers were also excluded because these tumors are thought to involve different etiologic pathways than tumors of other types (38).

Control subjects were selected from residents of the same geographic regions as the case patients, and they were frequency matched to the case patients by age (in 5-year age groups), race (African American or white; Hispanic women who reported their race as African American or white were eligible), and reference date (± 3 months). Control subjects could not have a history of endometrial cancer or hysterectomy. Selection of control subjects was carried out by a survey research firm, as previously described (37).

Additional eligibility criteria for both case patients and control subjects included living in a noninstitutional setting, having a household telephone, ability to speak English, and lacking severe cognitive, language, or speech impairment. To minimize the potential bias of selecting control subjects for the study from among individuals who are frequently at home and may differ from individuals who are frequently out of the house, we required nine attempts at contact at multiple times of the day and days of the week. The interval between diagnosis and case ascertainment could not exceed 18 months, and the interval between ascertainment and contacting case patients for the screening interview could not exceed 12 months.

Telephone interviews were used to collect data on demographic characteristics; family history of breast, endometrial, and ovarian cancer; contraceptive history; fertility history; menstrual and menopausal history; medical history; detailed gynecologic screening and history; and use of exogenous hormones, herbal preparations commonly used for menopausal symptoms, and other medications. All information on exposures pertained to a participant's history before the reference date, which was defined as the date of diagnosis for the case patients or the date of completion of the random-digit-dialing screening for the control sub-

jects. Eligible women were postmenopausal at the reference date. The study was approved by the University of Pennsylvania Committee on Studies Involving Human Beings and by the Institutional Review Boards of the 61 participating hospitals. Subjects gave verbal informed consent for the interview and written informed consent for the buccal samples.

Participation

We ascertained 1185 case patients with incident endometrial cancer who met the age, county, diagnostic, diagnosis date, menopausal status, and race criteria. Of these 1185 patients, 386 (32.6%) were excluded from further study because they were living in a nursing home ($n = 7$), did not speak English ($n = 29$), were not mentally or physically able to participate ($n = 17$), did not have physician consent ($n = 194$), did not have correct address and/or phone number ($n = 70$), or died before we could contact them for an interview ($n = 69$). Another 153 (12.9%) of them refused to participate, and 30 (2.5%) could not be interviewed before the study ended. Of the 799 case patients who were eligible and accessible, 616 (77.1%) were interviewed, which represents 52% of identified case patients. We excluded 37 (6.0%) of the 616 interviewed case patients with clear cell and papillary serous cancers from this analysis. Of the remaining 579 interviewed case patients with eligible histologic results, the 502 (86.7%) who also provided a DNA sample constituted the final sample of case patients in this study.

A total of 2708 potential control subjects were identified. Of these 2708, 405 (15.0%) were ineligible by the criteria of age, sex, county, race, menopausal status, or history of hysterectomy or of endometrial cancer. Of the 2303 control subjects remaining, 25 (1.1%) could not participate because of physical or mental impairment, 12 (0.5%) did not speak English, 7 (0.3%) were deceased, 207 (9.0%) could not be recontacted because they had moved or changed their phone number, and 469 (20.4%) refused. As a result, 1583 (68.7%) of eligible age- and race-matched control subjects completed the interview. Of these 1583, 1326 (83.8%) also provided a DNA sample and had at least one genotype that could be determined.

Laboratory Methods

Buccal swabs were obtained by mail from each participant. Genomic DNA was extracted with the QIAamp 96 DNA Buccal Swab Biorobot Kit by use of a 9604 Biorobot (Qiagen, Inc, Valencia, CA).

A total of 11 variants in nine genes (including single-nucleotide polymorphisms) were selected for study because of their roles in the downstream metabolism of steroid hormones (Table 1 and Fig. 1): COMT Val158Met (rs4680), CYP1A1 Ile462Val (*2C; rs1048943), CYP1A2*1F (rs762551), CYP1B1 Leu432Val (*3; rs1056836) and Asn452Ser (*4; rs1800440), CYP3A4*1B (rs2740574), PGR G \rightarrow A promoter variant at position 331 (PGR 331G>A; rs10895068), SULT1A1 Arg213His (*2; rs9282861) and Met223Val (*3; rs1801030), SULT1E1 G \rightarrow A promoter variant at position -64 (SULT1E1 -64G>A; rs3736599), and the UGT1A1 TA_n promoter repeat polymorphism. COMT, CYP1A1, CYP1A2, CYP1B1, CYP3A4, and SULT1E1 were assayed by use of Taqman (Applied Biosystems, Foster City, CA). Taqman 5' nuclease polymerase chain reaction (PCR) primers and probes for the alleles of interest were generated by the Assay-By-Design

Table 1. Allele and genotype frequencies by race and case-control status†

Gene	SNP nucleotide designation	SNP aliases, functional designations	Hypothesized variant function	White allele frequency		African American allele frequency	
				Case patients	Control subjects	Case patients	Control subjects
COMT	1947G>A	Val158Met, Val108Met	Met allozyme has decreased activity (50)	0.503	0.493‡	0.283	0.364
CYP1A1	6750A>G	Ile462Val, *2C, m2	Val allozyme has increased inducibility to produce 2-hydroxycatecholestrogens (51)	0.055	0.032‡	0.041	0.057
CYP1A2	734C>A or -163C>A	*1F	*1F allele has increased inducibility and ultrarapid activity to produce 2-hydroxycatecholestrogens (52)	0.305	0.275‡	0.391	0.398
CYP1B1	1294G>C or 4326C>G	Leu432Val, *3	Val allozyme has increased 4-hydroxy activity and increased adduct formation (53)	0.564	0.470	0.277	0.286‡
CYP1B1	1358A>G or 3290A>G	Asn452Ser, *4	Ser allozyme is associated with higher catalytic efficiency toward 4-hydroxyestradiol (53)	0.185	0.168	0.032	0.040
CYP3A4	729A>G	*1B	*1B allele may confer increased CYP3A4 expression (54)	0.029‡	0.050‡	0.587‡	0.614‡
PGR	331G>A	Promoter variant	A allele is associated with increased PGR transcription, favoring hPR-B production (55)	0.057	0.057	0.000	0.017
SULT1A1	638G>A	Arg213His, *2	*2 allozyme has lower thermostability, lower enzyme activity, and lower estrogen sulfation ability than wild-type enzyme (39,56–58)	0.302	0.303‡	0.250	0.252
SULT1A1	667A>G	Met223Val, *3	*3 allozyme has similar or lower activity than wild-type enzyme, depending on substrate (56–58)	0.019	0.030‡	0.193	0.270
SULT1E1	-64G>A	Promoter variant	Unknown	0.104	0.083‡	0.188	0.171
UGT1A1	TA _n	Promoter variants *1 (TA ₆) *28 (TA ₇) *33 (TA ₅) *34 (TA ₈)	*1 and *33 alleles have high activity and *28 and *34 alleles have low activity (59)	0.669 0.325 0.006 0.000	0.659 0.334 0.005 0.002	0.419 0.430 0.105 0.047	0.490 0.396 0.064 0.050

†SNP = single-nucleotide polymorphism; hPR-B = human progesterone receptor type B. Alleles are denoted by an asterisk and number.

‡Indicates deviations from Hardy-Weinberg proportions on the basis of a two-sided exact test with statistical significance at a *P* value of less than .05.

custom oligonucleotide reagent service (Applied Biosystems). Various DNA sequences were amplified by PCR, according to manufacturer's directions with MJ Research tetrad thermal cyclers (MJ Research Inc, Waltham, MA). Primer and probe sequences are available as Supplementary Data (available at <http://jncicancerspectrum.oxfordjournals.org/jnci/content/vol98/issue18>). After PCR, microtiter plates were transferred to the ABI 7900 HT Sequence Detection System. Endpoint fluorescence levels of 6-FAM and VIC were measured automatically in each well with the manufacturer's custom software SDS version 2.1 (ABI). Assays were also validated by use of direct sequencing (CYP1A1*2C), pyrosequencing (SULT1E1 -64G>A), and restriction fragment length polymorphism (RFLP; COMT, CYP1B1*3 and *4, and CYP3A4*1B). SULT1A1 genotypes were determined by use of a pyrosequencing assay, as previously reported (39).

The PGR 331G>A variant located 331 base pairs upstream from the transcription start site (412 base pairs upstream from the translation start site) was assessed with a PCR-RFLP assay. Oligonucleotide primers PGR331.F1 (5'-GTACGGAGCCAGCA GAAGTC-3') and PGR.R1 (5'-GAGGACTGGAGACGCAG AGT-3') were used in a PCR containing 17 µL of double-distilled H₂O, 20 µL of Eppendorf Master Mix, 5 µL of each primer at 5 µM, and 5 µL of template DNA in a total volume of 50 µL.

The PCR included 94 °C for 3 minutes, 35 cycles of 94 °C for 30 seconds, 59 °C for 30 seconds, and 72 °C for 30 seconds, followed by 72 °C for 7 minutes. Samples with weak PCR amplification underwent a nested PCR before RFLP analysis. The same primers as the original PCR were used with the following reaction conditions: 15 µL of double-distilled H₂O, 20 µL of Eppendorf Master Mix, 5 µL of each primer at 5 µM, and 5 µL of template DNA in a total volume of 50 µL. Template DNA for the nested PCR came from a 1:89 dilution of the original PCR product in double-distilled H₂O. The variant was detected by using *Nla*IV restriction endonuclease digestion that included, in the reaction mixture, 10 µL of PCR-amplified DNA, 0.25 µL of bovine serum albumin, 2.5 µL of New England Biolabs NEB buffer 4, 11.5 µL of double-distilled H₂O, and 1 µL of *Nla*IV at 1000 units/mL (New England Biolabs, Beverly, MA), incubated at 37 °C for 4 hours. Genotypes were visualized on a 3% Nusieve gel (Cambrex Bio Science, Rockland, ME) with ethidium bromide staining.

UGT1A1 was genotyped by use of pyrosequencing, as described by Saeki et al. (40). The UGT1A1 gene was amplified from genomic DNA by PCR with primers bTATA-F (5'-biotin-TCCCTGCTACCTTTGTGGAC-3') and UGT1A1-TATA-R (5'-GAGGTTTCGCCCTCTCCTACT-3'). The 25-µL amplification reaction mixture included 20 ng of total human genomic DNA as

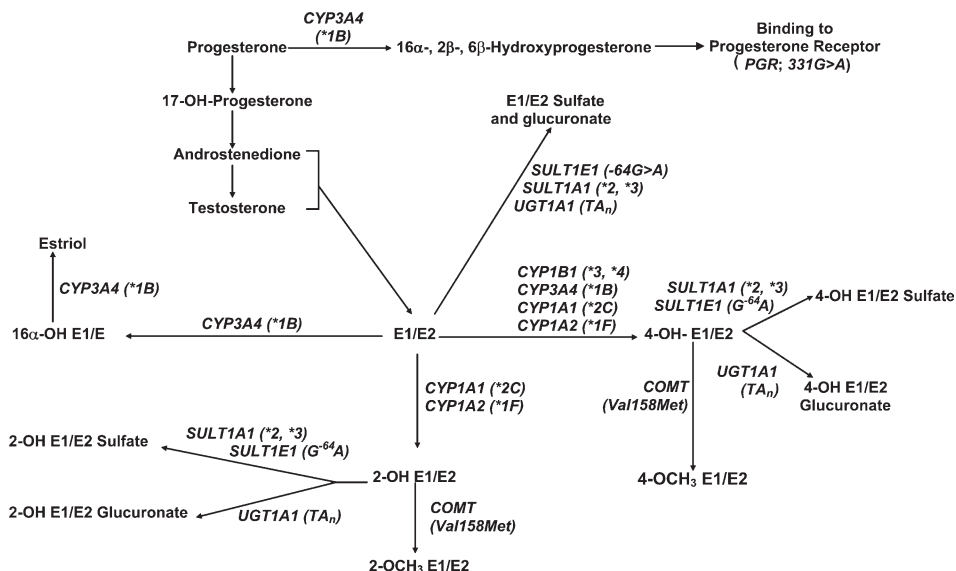


Fig. 1. Steroid hormone metabolism pathways with candidate genes. Genetic variants in the genes studied are shown in parentheses. E1 = estrone; E2 = estradiol.

template, 0.2 μM of each primer, all four deoxyribonucleoside triphosphates (each at 50 μM), 1.5 mM MgCl₂, and 0.5 U of Taq-Plus Precision DNA polymerase. Reaction conditions included initial denaturation for 2 minutes at 95 °C; followed by 35 cycles of 95 °C for 30 seconds, 52 °C for 30 seconds, and 72 °C for 30 seconds; followed by a final extension at 72 °C for 2 minutes. The products were visualized by 2% agarose gel electrophoresis at 100 V for 35 minutes. PCR fragments were subjected to pyrosequencing on the PSQ 96 with the pyrosequencing primer UGT1A1-TATA-SeqR (5'-TCGCCCTCTCCTACTTATAT-3') (40). Genotypes were assigned as UGT1A1*33, UGT1A1*1, UGT1A1*28, or UGT1A1*34 for five, six, seven, or eight TA repeats, respectively.

Statistical Methods

Odds ratio (OR) estimates and 95% confidence intervals (CIs) were calculated to evaluate the relationship of hormone metabolism genes and hormone replacement therapy use to the risk of endometrial cancer. Use of hormone replacement therapy was classified as 1) short-term use of estrogen replacement therapy (<3 years) versus never use of any hormone replacement therapy, 2) long-term use of estrogen replacement therapy (≥3 years) versus never use of any hormone replacement therapy, 3) short-term use of combined hormone replacement therapy (<3 years) versus never use of any hormone replacement therapy, and 4) long-term use of combined hormone replacement therapy (≥3 years) versus never use of any hormone replacement therapy. Use of any estrogen replacement therapy was defined as exposure to estrogen replacement therapy regardless of whether there were other periods of combined hormone replacement therapy or unopposed progestin use. Use of any combined hormone replacement therapy was defined as exposure to combined hormone replacement therapy regardless of whether there were other periods of estrogen replacement therapy or unopposed progestin use.

Our goal was to include only postmenopausal women in our study. Although we used objective criteria to determine menopausal status for most women, menopausal status could not be determined for some women. Therefore, we used age cutpoints to

determine menopausal status. For the present analysis, we considered women older than 50 years to be postmenopausal if we had no other information on which to base this decision. Because this cutpoint may include some women older than 50 years who were pre- or perimenopausal, we undertook a sensitivity analysis with a cutpoint defining women as postmenopausal if older than 55 years. This cutpoint was overly conservative (i.e., excluded some postmenopausal women from our analysis). However, there were no differences in the inferences related to hormone replacement therapy exposures that depended on the age cutpoint used (14).

Genotype was coded based on knowledge of the functional effects of the variants, as well as the frequency of genotypes of interest (Table 1). Therefore, the genotype classes that we considered reflect functionally relevant classes (when known) that provided sufficient statistical power for associations on the basis of our study design. To identify potential genotyping errors and deviations from Hardy–Weinberg proportions, exact tests were performed in the STATA computer program by use of the “genhwi” command.

Multiple conditional logistic regression was used to simultaneously account for the matching variables of age (in 5-year age groups), race (African American or white), and reference date (±3 months). To account for additional confounding, models were also adjusted for the same set of variables: education (<high school graduate, high school graduate, >high school but <college graduate, or ≥college graduate), body mass index as a continuous variable from age 40 years through 49 years, number of full-term pregnancies (never pregnant or 1, 2, or ≥3), years of menses as a continuous variable, menopause type (known natural, assumed natural at reference age of 50 years if menopausal status is unknown, or induced), smoking status (never smoker or former or current smoker × years of smoking), and oral contraceptive use (never, <3 years, or ≥3 years). Additional variables measured as potential confounders were evaluated to assess whether they changed the point estimates of relevant genotypic effects by 10% or more. None of these variables met these criteria, and therefore, none was included in the models.

To evaluate interactions between all candidate genotypes and these hormone exposures, two sets of primary a priori hypotheses

were specified. First, we evaluated whether there was an association of each genotype adjusted for hormone use and the other covariates described above. Second, we assessed whether the association between hormone replacement therapy exposure and risk depended on genotype by use of a likelihood ratio test with 2 df; i.e., we compared the effects of a short-term (<3 years) and long-term (≥3 years) exposure between those in the reference and variant genotype groups. Also, to account for the possibility that use of hormone replacement therapy might obscure the onset of natural menopause, we required no use of hormone replacement therapy at any time before the last menstrual period.

Because we have performed multiple hypothesis tests, we considered the potential for false-positive findings. Accordingly, we applied the false-positive report probability approach (41), an approach that allows the investigator to interpret the results of hypothesis testing to ensure against making false-positive inferences. We considered 12 a priori hypotheses corresponding to the 12 genetic variant groupings tested (including two hypotheses for the discrete genotype classes constructed for UGT1A1 genotypes; Table 1). All analyses were performed in STATA (version 9.0, STATA Corporation, College Station, TX). All statistical tests were two-sided.

RESULTS

As shown in Table 2, case patients with endometrial cancer were older than the control subjects overall (63.0 versus 61.3 years, $P < .001$) because of the race-specific matching algorithm (3) and had a higher percentage of never-married women (8% versus 6%, $P = .041$). Of the 502 case patients, 447 (89%) were white and 55 (11%) were African American. The case patients had more years of schooling than the control subjects (47% versus 55% without schooling beyond high school; $P = .001$) and higher income (43% versus 49% with income \$45 000 or less; $P = .009$). Many of the known risk factors for endometrial cancer were more commonly observed in case patients than in control subjects, including obesity (22% versus 10% had a body mass index of ≥ 30 kg/m²; $P < .001$), diabetes (19% versus 11%; $P < .001$), hypertension (52% versus 44%; $P < .001$), the presence of gallbladder and gallstone problems (22% versus 15%; $P < .001$), younger age at menarche (28% versus 21% underwent menarche at <12 years; $P < .001$), older age at menopause (64% versus 55% underwent menopause at >50 years; $P < .001$), nulliparity (17% versus 10%; $P < .001$), and family history of endometrial cancer (8% versus 5%; $P = .012$). In addition, many of the known or suggested factors that are associated with lower risk for endometrial cancer were less prevalent among case patients than among control subjects, including ever use of combined oral contraceptives (41% versus 56%; $P < .001$), past or current history of cigarette smoking (49% versus 58%; $P < .001$), osteoporosis (15% versus 19%; $P = .038$), tubal ligation (24% versus 33%; $P < .001$), younger age at first pregnancy (11% versus 17% were <20 years; $P = .002$), older age at last pregnancy (14% versus 21% were ≥ 35 years; $P < .001$), and ever breastfeeding (35% versus 43%; $P < .001$). These results demonstrate that our data are consistent with previous reports regarding endometrial cancer risk factors. Because of these observed case-control differences, we considered these factors as potential confounding variables in our primary analyses of the relationship between genotype and the risk of endometrial cancer.

Table 2. Demographic characteristics of postmenopausal case patients with endometrial cancer and matched control subjects in the Philadelphia area, 1999–2002

Characteristic	Case patients	Control subjects	P value
	(n = 502)	(n = 1326)	
Age at reference date, y	63.0	61.3	<.001
Race			<.001
White	447 (89)	1002 (76)	
Black	55 (11)	324 (24)	
Marital status			.041
Never married	40 (8)	80 (6)	
Ever married	461 (92)	1243 (94)	
Unknown	1 (<1)	3 (<1)	
Highest schooling			.001
Less than high school	236 (47)	729 (55)	
Greater than high school	266 (53)	595 (45)	
Unknown	0	2 (<1)	
Household income (pretax)			.009
≤\$45 000	216 (43)	650 (49)	
>\$45 000	211 (42)	530 (40)	
Unknown	0	2 (<1)	
Body mass index*			<.001
<30 kg/m ²	387 (77)	1192 (90)	
≥30 kg/m ²	110 (22)	133 (10)	
Unknown	5 (1)	1 (<1)	
History of diabetes (ever)	95 (19)	146 (11)	<.001
Hypertension (ever)	261 (52)	583 (44)	<.001
Gallbladder problems/ gallstones (ever)	110 (22)	199 (15)	<.001
Osteoporosis	75 (15)	252 (19)	.038
Tubal ligation	120 (24)	438 (33)	<.001
Age at first menarche			<.001
Early (≤12 y)	141 (28)	278 (21)	
Late (≥13 y)	359 (72)	1042 (79)	
Unknown	2 (<1)	6 (<1)	
Imputed Age at Menopause†			<.001
Early (≤50 y)	181 (36)	597 (45)	
Late (>50 y)	321 (64)	729 (55)	
Never pregnant	85 (17)	133 (10)	<.001
Age at first full-term‡ pregnancy			.002
Never pregnant	85 (17)	133 (10)	
<20 y	55 (11)	225 (17)	
≥20 y	362 (72)	964 (73)	
Unknown	0	4 (<1)	
Age at last full-term‡ pregnancy			<.001
Never pregnant	85 (17)	133 (10)	
<35 years old	347 (69)	911 (69)	
≥35	70 (14)	278 (21)	
Unknown	0	4 (<1)	
Breastfeeding			<.001
Never	325 (65)	755 (57)	
Ever	176 (35)	570 (43)	
Unknown	1 (<1)	1 (<1)	
Combined oral contraceptive use			<.001
Never used	296 (59)	579 (44)	
Ever used	206 (41)	743 (56)	
Unknown	0	4 (<1)	
Cigarette smoker			<.001
Never	256 (51)	555 (42)	
Ever	246 (49)	769 (58)	
Unknown	0	2 (<1)	
Family history of endometrial cancer (in mother, sisters, daughters)	40 (8)	66 (5)	.012

*Body mass index is based on a woman's usual weight between the ages of 40 years and 49 years.

†Age at menopause was imputed where unknown from age at first use of menopausal hormone replacement therapy, if available.

‡Defined as a pregnancy lasting longer than 26 weeks.

Table 3. Adjusted odds ratios (ORs) for endometrial cancer by main effect of genotype and unopposed estrogen replacement therapy (ERT) or combined estrogen–progestin replacement therapy (CHRT) use†

Factor	Variant or exposure vs. reference	Reference (No. case patients/No. control subjects)	Variant or exposure (No. case patients/No. control subjects)	OR (95% CI)
ERT use	<3 y vs. never	385/938	44/140	0.91 (0.60 to 1.37)
	≥3 y vs. never	385/938	45/31	2.41 (0.41 to 4.13)
CHRT use	<3 y vs. never	385/938	51/185	0.86 (0.58 to 1.27)
	≥3 y vs. never	385/938	81/264	0.92 (0.66 to 1.28)
COMT	Met/Met vs. other	367/990	117/247	1.21 (0.89 to 1.64)
CYP1A1	Any *2C vs. none	450/1231	52/95	1.68 (1.08 to 2.61)
CYP1A2	Any *1F vs. none	50/126	425/1107	1.08 (0.71 to 1.66)
CYP1B1*3	Any *3 vs. none	119/376	371/877	1.11 (0.83 to 1.49)
CYP1B1*4	Any *4 vs. none	339/956	144/320	1.05 (0.80 to 1.39)
CYP3A4	Any *1B vs. none	425/980	57/328	0.66 (0.41 to 1.05)
PGR	A/A or A/G vs. G/G	441/1165	48/110	1.02 (0.68 to 1.54)
SULT1A1*2	Any *2 vs. none	216/532	205/491	1.03 (0.78 to 1.35)
SULT1A1*3	Any *3 vs. none	392/863	29/160	0.51 (0.29 to 0.92)
SULT1E1	A/A or A/G vs. G/G	393/1061	103/245	1.45 (1.06 to 1.99)
UGT1A1	Low/high vs. high/high	182/636	201/466	1.06 (0.80 to 1.42)
	Low/low vs. high/high	182/636	45/156	0.83 (0.53 to 1.29)

†Odds ratios (with 95% confidence intervals [CIs]) were estimated with conditional logistic regression analysis, in which control subjects were matched to case patients by age and race, and adjusted for the following variables: education (<high school [HS], HS graduate, >HS but <college graduate, or >college graduate); body mass index from age 40 years through 49 years as a continuous variable; number of full-term pregnancies (never pregnant or 1–2 or ≥3); years of menses, imputed if missing, as a continuous variable; type of menopause (known natural, assumed natural at reference age of 50 years if menopausal status is unknown, or induced); interaction of never, former, or current smoker by years of smoking; and oral contraceptive use (never, <3 years, or ≥3 years). Alleles are denoted by an asterisk and number.

Overall use of hormone replacement therapy by the postmenopausal women in our study was 45%. Use of hormone replacement therapy was much lower among African American women than among white women. Among the 379 African American women, 41 (11%) had used any estrogen replacement therapy for any duration and 54 (14%) had used any combined hormone replacement therapy for any duration. Among 1449 white women, 265 (18%) had used any estrogen replacement therapy of any duration and 509 (35%) had used any combined hormone replacement therapy of any duration.

The main effects of each genotype and the statistical interactions between genotypes and exogenous hormone exposures are presented in Table 3. The other confounders considered in all models were education, body mass index, number of full-term pregnancies, years of menses, type of menopause, smoking status, and history of oral contraceptive use. As previously reported (14), long-term (≥3 years) estrogen replacement therapy use was statistically significantly associated with endometrial cancer risk (adjusted OR = 3.4, 95% CI = 1.4 to 8.3). Combined hormone replacement therapy exposure of any duration was not associated with risk (adjusted OR = 0.90, 95% CI = 0.67 to 1.19). These main effects were similar in all analyses, including those that included interaction terms involving estrogen replacement therapy or combined hormone replacement therapy.

Genotyping success rates varied across the variants studied, with failure rates ranging from 5.6% to 12.5% and most having failure rates of less than 10%. Deviations from Hardy–Weinberg proportions were observed in some groups defined by case status and race (Table 1), but these deviations were not consistent across all groups for a particular genotype, suggesting that genotyping error was not the cause of these deviations. The only genotype for which Hardy–Weinberg deviations were observed was CYP3A4*1B, which deviated from Hardy–Weinberg proportions in case patients and control subjects of both races. This result is consistent with deviations from Hardy–Weinberg equilibrium that have been reported in many studies of this variant and re-

flects the fact that this variant deviates from Hardy–Weinberg equilibrium in most populations (42).

As shown in Table 3, no association was observed between endometrial cancer risk and genotype for COMT, CYP1A2, CYP1B1, CYP3A4, PGR, SULT1A1*2, or UGT1A1 genes. However, statistically significant associations were observed with genotypes that contained any CYP1A1*2C allele, compared with genotypes without a *2C allele (adjusted OR = 1.68, 95% CI = 1.08 to 2.61); with genotypes that contained any allele for the SULT1E1 –64G>A polymorphism, compared with genotypes without such an allele (adjusted OR = 1.45, 95% CI = 1.06 to 1.99); and with genotypes that contained any SULT1A1*3 allele, compared with genotypes without such an allele (adjusted OR = 0.51, 95% CI = 0.29 to 0.92).

We observed a statistically significant interaction between use of estrogen replacement therapy and SULT1A1*2 genotype (Table 4), indicating that the effect of estrogen replacement therapy use differed according to the individual's SULT1A1*2 variant genotype status. Among subjects with the reference genotype (SULT1A1*1), risk of endometrial cancer was not associated with use of estrogen replacement therapy of less than 3 years or 3 years or more. However, among subjects with the variant genotype (SULT1A1*2), use of estrogen replacement therapy for less than 3 years was associated with a decreased risk of endometrial cancer (OR = 0.40, 95% CI = 0.18 to 0.88) and use for 3 years or more was associated with a statistically significantly increased risk (OR = 3.85, 95% CI = 1.48 to 10.00).

As shown in Table 4, the global (2 df) test assessing the interaction between genotype and use of hormone replacement therapy was statistically significant ($P = .011$), indicating that at least one of the two odds ratios for estrogen replacement therapy use (either short term or long term) differed between those with and without the SULT1A1*2 variant. We also considered models that included use of other hormone replacement therapy (i.e., use of unopposed progesterone or other forms of hormone replacement therapy not included in our definition of estrogen replacement

Table 4. Adjusted odds ratios (ORs with 95% confidence intervals [CIs]) for endometrial cancer by genotype and by use of unopposed estrogen replacement therapy (ERT)†

Gene	Variant vs. reference genotype comparison	OR (95% CI) among women with reference genotype		OR (95% CI) among women with variant genotype	
		<3 y ERT use vs. never	≥3 y ERT use vs. never	<3 y ERT use vs. never	≥3 y ERT use vs. never
COMT	Met/Met vs. other	0.94 (0.56 to 1.57)	2.17 (1.08 to 4.36)	0.66 (0.25 to 1.74)	2.73 (0.95 to 7.86)
CYP1A1	Any *2C vs. none	0.89 (0.56 to 1.42)	2.49 (1.40 to 4.42)	0.52 (0.13 to 2.11)	–‡
CYP1A2	Any *1F vs. none	0.15 (0.02 to 1.34)	2.69 (0.45 to 16.22)	1.04 (0.65 to 1.67)	2.46 (1.32 to 4.60)
CYP1B1*3	Any *3 vs. none	1.18 (0.52 to 2.67)	2.10 (0.55 to 8.03)	0.81 (0.47 to 1.40)	2.65 (1.41 to 5.01)
CYP1B1*4	Any *4 vs. none	0.76 (0.44 to 1.35)	1.62 (0.78 to 3.34)	1.18 (0.54 to 2.61)	5.10 (1.97 to 13.20)
CYP3A4	Any *1B vs. none	0.89 (0.55 to 1.44)	2.88 (1.56 to 5.32)	0.37 (0.04 to 3.04)	2.15 (0.43 to 10.84)
PGR	A/A or A/G vs. G/G	0.88 (0.55 to 1.42)	1.98 (1.07 to 3.67)	0.93 (0.22 to 3.94)	3.26 (0.53 to 20.10)
SULT1A1*2	Any *2 vs. none	1.47 (0.74 to 2.92)§	1.87 (0.78 to 4.49)§	0.40 (0.18 to 0.88)§	3.85 (1.48 to 10.00)§
SULT1A1*3	Any *3 vs. none	0.76 (0.46 to 1.29)	2.65 (1.37 to 5.12)	0.86 (0.09 to 7.85)	–‡
SULT1E1	A/A or A/G vs. G/G	0.97 (0.58 to 1.63)	2.89 (1.53 to 5.43)	0.71 (0.28 to 1.80)	0.76 (0.17 to 3.49)
UGT1A1	Low/high vs. high/high	0.95 (0.46 to 1.95)	2.41 (0.97 to 5.99)	0.60 (0.27 to 1.32)	2.31 (0.90 to 5.92)
	Low/low vs. high/high	0.98 (0.47 to 2.04)	2.52 (1.01 to 6.27)	1.20 (0.38 to 3.85)	1.79 (0.16 to 20.02)

†Odds ratios were estimated from conditional logistic regression analysis, in which control subjects were matched to case patients by age and race, and adjusted for the following variables: education (<high school [HS], HS graduate, >HS but <college graduate, or >college graduate); body mass index from age 40 years through 49 years as a continuous variable; number of full-term pregnancies (never pregnant, 1–2, or ≥3); years of menses, imputed if missing, as a continuous variable; type of menopause (known natural, assumed natural at reference age of 50 years if menopausal status is unknown, or induced); interaction of never, former, and current smoker by years of smoking; oral contraceptive use (never, <3 years, or ≥3 years). The odds ratios compare women with the specified use of ERT to women who never used hormone replacement therapy, among those with the specified genotype. Alleles are denoted by an asterisk and number.

‡Sample size was insufficient to obtain reliable estimates.

§Two-sided likelihood ratio test with 2 df for interaction that was statistically significant at a *P* value of .011.

therapy or combined hormone replacement therapy). The interactions remained statistically significant whether we included or excluded the use of other hormone replacement therapy in the model. Additional 1 df tests of interactions for duration of estrogen replacement therapy use and for genotype assisted in clarification of the source of this difference. First, for subjects with short-term exposure, the effect of genotype was not associated with endometrial cancer ($\chi^2_1 = 2.59$ and $P = .207$), when the adjusted odds ratio estimates of 1.47 and 0.40 in Table 4 were compared. Second, for subjects with long-term exposure, the effect of genotype was statistically significantly associated with endometrial cancer ($\chi^2_1 = 7.41$ and $P = .007$), when the adjusted odds ratio estimates of 1.87 and 3.85 in Table 4 are compared. Third, for subjects with the reference genotype, the effect of length of estrogen replacement therapy use was not statistically significantly associated with endometrial cancer risk ($\chi^2_1 = 0.13$ and $P = .720$), when the adjusted odds ratio estimates of 1.47 and 1.87 in Table 4 were compared. Finally, for subjects with the variant genotype, the effect of length of estrogen replacement therapy use was statistically significantly associated with risk ($\chi^2_1 = 15.98$ and $P < .001$), when the adjusted odds ratio estimates of 0.40 and 3.85 in Table 4 were compared.

To evaluate the possibility that the statistically significant associations reported in Tables 4 and 5 represent false-positive findings, we calculated the false-positive report probability (41) for relevant associations in Tables 4 and 5 by assuming prior probabilities of an association among genotype, hormone use, and endometrial cancer of .05, .1, and .2. For all main effect odds ratios that we reported as statistically significantly different from an odds ratio of 1.0 in Tables 4 and 5, the false-positive report probability was less than .3 (and generally less than .2) unless the prior probability was low (i.e., ≤.05). For the effects of estrogen replacement therapy use among those with the variant SULT1A1 gene, a false-positive report probability of .3 or less was observed when the prior probability of an association was assumed to be .05 or more. That is, these estimated odds ratios

can be considered likely to be true-positive associations only when the prior probability for this association is assumed to be .05 or more. Given the strong biologic plausibility that SULT1A1 could be involved in mediating the biologic effect of estrogen replacement therapy use, it is reasonable to make such an assumption.

DISCUSSION

We have reported that variants in CYP1A1, SULT1E1, and possibly SULT1A1 genes are associated with endometrial cancer risk. We have also reported that the statistical interaction of SULT1A1 genotypes with the use of estrogen replacement therapy appears to influence endometrial cancer risk. Inferences from most of the literature, including the present study, indicate that use of estrogen replacement therapy is associated with an increased risk of endometrial cancer and that use of combined hormone replacement therapy is inversely associated with the risk of endometrial cancer (8,11–17,19–22). Our results further indicate that these effects may differ according to whether women carry the functional SULT1A1*2 variant.

Genes involved in the sulfation of estrogens have not been previously studied in the context of endometrial cancer etiology. We identified an association between the –64G>A variant in SULT1E1 and endometrial cancer risk and showed that the association of estrogen replacement therapy and endometrial cancer risk was different depending on SULT1A1*2 genotype. Sulfation by SULT1A1 and SULT1E1 enzymes may protect endometrial cells from the mitogenic and DNA-damaging activities of estradiol and its catecholesterol metabolites (43). Sulfation of 2-hydroxycatecholesterol can also compete with its methylation (Fig. 1). This methylated product is antimutagenic and antiproliferative (44,45). SULT1E1 has high affinity for estrone, estradiol, and catecholestrogens (46). However, the function of the SULT1E1 –64G>A variant is not yet known, so we cannot infer whether this association is biologically meaningful,

Table 5. Odds ratios (ORs with 95% confidence intervals [CIs]) associated with genotype and use of combined estrogen and progestin replacement therapy (CHRT) in endometrial cancer†

Gene	Variant vs. reference genotype comparison	OR (95% CI) among women with reference genotype		OR (95% CI) among women with variant genotype	
		<3 y CHRT use vs. never	≥3 y CHRT use vs. never	<3 y CHRT use vs. never	≥3 y CHRT use vs. never
COMT	Met/Met vs. other	0.76 (0.46 to 1.26)	0.70 (0.46 to 1.06)	0.94 (0.43 to 2.10)	1.37 (0.68 to 2.77)
CYP1A1	Any *2C vs. none	0.75 (0.48 to 1.17)	0.76 (0.52 to 1.10)	0.88 (0.23 to 3.45)	1.38 (0.42 to 4.61)
CYP1A2	Any *1F vs. none	0.65 (0.28 to 2.36)	0.63 (0.20 to 2.02)	0.89 (0.56 to 1.41)	0.83 (0.56 to 1.22)
CYP1B1*3	Any *3 vs. none	0.71 (0.31 to 1.65)	0.76 (0.36 to 1.59)	0.80 (0.49 to 1.31)	0.84 (0.56 to 1.25)
CYP1B1*4	Any *4 vs. none	0.85 (0.52 to 1.41)	0.67 (0.43 to 1.04)	0.63 (0.28 to 1.41)	1.10 (0.60 to 2.03)
CYP3A4	Any *1B vs. none	0.79 (0.49 to 1.26)	0.86 (0.59 to 1.26)	0.45 (0.12 to 1.72)	0.54 (0.16 to 1.81)
PGR	A/A or A/G vs. G/G	0.76 (0.47 to 1.21)	0.75 (0.41 to 1.10)	2.02 (0.58 to 7.02)	2.44 (0.89 to 6.69)
SULT1A1*2	Any *2 vs. none	0.96 (0.49 to 1.88)	0.69 (0.40 to 1.19)	0.79 (0.41 to 1.54)	1.08 (0.63 to 1.84)
SULT1A1*3	Any *3 vs. none	0.82 (0.49 to 1.35)	0.88 (0.59 to 1.31)	3.19 (0.64 to 15.92)	–‡
SULT1E1	A/A or A/G vs. G/G	0.86 (0.54 to 1.38)	0.84 (0.56 to 1.24)	0.36 (0.10 to 1.34)	0.83 (0.38 to 1.83)
UGT1A1	Low/high vs. high/high	0.79 (0.39 to 1.63)	1.01 (0.58 to 1.76)	0.96 (0.49 to 1.88)	0.99 (0.56 to 1.73)
	Low/low vs. high/high	0.74 (0.35 to 1.55)	0.99 (0.56 to 1.74)	0.66 (0.19 to 2.31)	0.40 (0.12 to 1.32)

†Odds ratios were estimated from conditional logistic regression analysis, in which control subjects were matched to case patients by age and race, and adjusted for the following variables: education (<high school [HS], HS graduate, >HS but <college graduate, or >college graduate); body mass index from age 40 years through 49 years as a continuous variable; number of full-term pregnancies (never pregnant or 1–2, or ≥3); years of menses, imputed if missing, as a continuous variable; type of menopause (known natural, assumed natural at reference age of 50 years if menopausal status is unknown, or induced); interaction of never, former, or current smoker by years of smoking; oral contraceptive use (never, <3 years, or ≥3 years). The odds ratios compare women with the specified use of estrogen replacement therapy to women who never used hormone replacement therapy, among those with the specified genotype. Alleles are denoted by an asterisk and number.

‡Sample size was insufficient to obtain reliable estimates.

occurred by chance, or reflects linkage disequilibrium with another genetic variant at or near SULT1E1.

We have also reported that the association between estrogen replacement therapy and endometrial cancer may be different according to SULT1A1*2 genotype. The biologic function of the SULT1A1*2 allele has been well characterized; the protein coded by the SULT1A1*2 genotype is associated with lower enzyme thermostability, lower enzyme activity (47), and lower estrogen sulfation ability than the nonvariant form (39,58). The SULT1A1 enzyme acts on ethinyl estradiol, estradiol, and catecholestrogens (Fig. 1). Thus, the decreased ability to sulfate exogenous estradiol that is associated with the use of estrogen replacement therapy may explain the increased risk of endometrial cancer in some women and be a possible mechanism for the development of endometrial cancer.

These data have potential clinical implications for the use of hormone replacement therapy. Because estrogen replacement therapy is strongly associated with an increased risk of endometrial cancer, until now it has been thought that unopposed estrogen replacement therapy should not normally be recommended for women with an intact uterus. Our results suggest that women who do not carry a SULT1A1*2 allele, and therefore have increased estrogen sulfation capacity, may not be at elevated risk of endometrial cancer when using long-term unopposed estrogen replacement therapy. Therefore, our results provide initial evidence that the risk of endometrial cancer associated with estrogen replacement therapy or combined hormone replacement therapy may in part depend on estrogen sulfation genotypes of the women who use these hormones.

We also evaluated other genes involved in the metabolic pathways of steroid hormones. No association was observed between endometrial cancer risk and COMT, CYP1A2, CYP1B1, CYP3A4, PGR, SULT1A1*3, or UGT1A1 genotype. Therefore, our results are consistent with reported lack of associations with CYP1A2 (32), COMT (30,32), and CYP1B1 (30–32), but they are inconsistent with previous reports of associations with CYP1B1 in a Japanese population (33) and with UGT1A1 (36)

and PGR (35). We also have reported an increased endometrial cancer risk associated with the CYP1A1*2C allele (i.e., Ile462Val), contrary to the findings of Doherty et al. (32), who reported that this polymorphism is inversely associated with endometrial cancer. Because of these discrepancies, we cannot confirm the association between these genes and endometrial cancer risk.

Despite the strength and biologic plausibility of most of the associations observed in this study, our study has some limitations. First, we specified a large number of a priori hypothesis tests, and it is thus possible that we have detected false-positive associations. We have computed the false-positive report probability of Wacholder et al. (41) to aid in interpreting our findings. In situations in which we observed statistically significant associations (Table 2), the false-positive report probability results generally supported these inferences. Another concern is that our study sample contained both African Americans and whites, leading to the potential for confounding by ethnicity (population stratification). However, we matched our case patients and control subjects by race, thus removing the majority of the variability that could lead to this confounding (48). We have also systematically evaluated the potential for bias in the estimates from logistic regression in case-control studies that involve admixed populations (49). In these evaluations, we did not identify substantial biases in the odds ratios, given the range of genotype frequencies and baseline disease risks that we found. In addition, we evaluated whether there were race-specific differences in the direction or magnitude of effects of genotype and hormone replacement therapy use by testing for the presence of interactions by race. No such interactions were observed, suggesting that the estimates of genotype and/or hormone replacement therapy effect were not statistically significantly different by race. Thus, the odds ratios presented in Table 2 are unlikely to be even moderately biased by population stratification. Although we have limited our analyses to evaluate first-order interactions between genotypes and hormone replacement therapy use, the sample sizes for the joint effects of these factors sometimes were very

small. Therefore, we may not have the power to detect some important effects in groups for which the joint genotype–hormone replacement therapy effects were small. Finally, the overall response rates among eligible participants in our study were low, although they were typical for population-based case–control studies of this type. The low response rates could have affected our results, particularly if the reasons for nonresponse were related to important risk factors or confounders involved in endometrial cancer etiology and/or if the nonresponse was differential with respect to case–control status. We have no evidence that this was the case, although we hypothesized that it may be less likely for bias of this type to influence genotype effects or interactions involving genotypes and exposures. But we cannot rule out bias toward or away from the null from this source.

In summary, we have reported novel associations between endometrial cancer risk and genotypes in the *SULT1A1* and *SULT1E1* genes. These results may help to identify important pathways to consider in the etiology of endometrial cancer. In addition, we report that endometrial cancer risk associated with use of estrogen replacement therapy may vary depending on genotypes in the steroid hormone metabolism gene *SULT1A1*. Although the risk of endometrial cancer associated with hormone replacement therapy appears to be influenced by genotypes that functionally alter the actions of the progesterone receptor or estrogen sulfation by the *SULT1A1* enzyme, additional work is required to determine whether this information is clinically useful and whether genetic testing for these genes may be warranted for women who consider exogenous hormone replacement.

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NOTES

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