The Functional UGT1A1 Promoter Polymorphism Decreases Endometrial Cancer Risk

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ABSTRACT

UDP-glucuronosyltransferase (UGT) 1A1 is involved in the inactivation of estradiol (E2) and its oxidized metabolites. These metabolites have been shown to contribute to the development of endometrial cancer in animal studies. Thus UGT1A1 represents a candidate gene in endometrial carcinogenesis. In this study, we established the substrate specificity of UGT1A1 for E2 and its 2- and 4-hydroxylated metabolites. Intrinsic clearances indicated that UGT1A1 had a preference for the glucuronidation of 2-hydroxysteriadiol, a metabolite associated with antiproliferative activity. Expression analysis demonstrated that UGT1A1 is present in the nonmalignant endometrium. Subsequently, we sought to determine whether the common UGT1A1 promoter allele, UGT1A1*28 [A(TA)]7TAA, which decreases gene transcription, was associated with endometrial cancer risk in a case-control study nested within the Nurses’ Health Study (222 cases, 666 matched controls). Conditional logistic regression demonstrated a significant inverse association with the UGT1A1*28 allele and endometrial cancer risk. Compared with women homozygous for the UGT1A1*1 [A(TA)]7TAA allele, the adjusted odds ratio (OR) was 0.81 (95% confidence interval [CI], 0.56–1.16) for the UGT1A1*1*28 genotype and 0.40 (95% CI, 0.21–0.75) for the homozygous UGT1A1*28 genotype (P = 0.007). There was a suggestion of an interaction by menopausal status [OR = 0.39 (95% CI, 0.18–0.85) for premenopausal women and OR = 0.79 (95% CI, 0.55–1.35) for postmenopausal women who carry the UGT1A1*28 allele (Pinteraction = 0.05)]. These observations suggest that lower expression of UGT1A1 decreases the risk of endometrial cancer by reducing the excretion of 2-hydroxyestradiol, the antiproliferative metabolite of E2, in the endometrium.

INTRODUCTION

Adenocarcinoma of the endometrium is the most common carcinoma of the female reproductive organs and ranks fourth in total cancer incidence among women in the United States (1). Biological evidence supports a role for estrogen as an endometrial carcinogen (2–4). Estrogen is metabolized by either formation of 4-hydroxyestradiol (4-OH-E2) and 2-hydroxyestradiol (2-OH-E2) or by C-16 hydroxylation. 4-OH-E2 is the most carcinogenic estrogen and induces uterine adenocarcinoma in mice (5). 2-OH-E2, which is conjugated to glucuronide, is less carcinogenic than its parent steroid (6). Evidence that estrogen metabolites are involved in the development of endometrial cancer has been isolated, and several have demonstrated significant reactivity toward estrogens and their metabolites, catecholestrogens (14–21). Estrogen is metabolized by either formation of 4-hydroxyestrogens, which are metabolized to 2-hydroxyestrogens and other metabolites, or by C-16 hydroxylation. 4-OH-E2 is the most carcinogenic estrogen and induces uterine adenocarcinoma in mice (5). 2-OH-E2, which is conjugated to glucuronide, is less carcinogenic than its parent steroid (6).

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and metabolism may be potential markers of endometrial cancer susceptibility (9–12).

UDP-glucuronosyltransferases (UGTs), a family of phase II metabolizing enzymes involved in estrogen biotransformation, have not been studied in relation to endometrial cancer risk. UGTs contribute to the maintenance of intracellular hormone homeostasis in a number of steroid hormone target tissues (13–16). Steroid hormones are conjugated with glucuronic acid by UGTs, rendering them more polar than their parent steroids. The addition of the sugar moiety prevents the steroids from binding to their receptor resulting in a loss of biological activity. In humans, more than 18 functional UGTs have been isolated, and several have demonstrated significant reactivity toward estrogens and their metabolites, catecholestrogens (14–21).

Glucuronidation presumably blocks the 4-hydroxy-catecholestrogens (4-OH-CEs) oxidation to quinone estrogens, reducing their mutagenic potential (3, 15, 22). In contrast to 4-OH-CE, the 2-hydroxy-catecholestrogens (2-OH-CE) metabolites lack carcinogenic potential (6, 23).

In addition, metabolic conversion of 2-OH-CE to 2-methoxy-catechol-estrogen (2-MeO-CE) by catechol-O-methyltransferase is a significant metabolic process in steroid target tissues (24–27). O-Methylation of 2-OH-CE to 2-MeO-CE results in the formation of a very potent inhibitor of cell proliferation, tubulin activity, and angiogenesis (8, 28, 29). As a result, glucuronidation of 2-OH-CEs, a detoxification process that prevents the formation of 2-MeO-CEs, would result in an increased cancer risk. UGT1A1 has a wide tissue distribution, however, its expression in endometrial tissue has not been investigated; as a result, its specific role in the metabolism of estrogen in the uterus remains unknown. We hypothesized that UGT1A1 plays a critical role in the catabolism of estrogens and catecholestrogens in the uterus affecting endometrial cancer risk.

In this study, our aims were 3-fold: to determine the relative catalytic activities of UGT1A1 for E2, 4-OH-E2, and 2-OH-E2 using a catalytic in vitro cell-based system to establish the glucuronidation of 2-OH-E2 and 4-OH-E2 by UGT1A1; to examine the expression of UGT1A1 in the endometrium; and to investigate the association between the known functional polymorphism in the promoter region of the UGT1A1 gene and endometrial cancer risk. A common variant in the UGT1A1 gene, designated as UGT1A1*28 [A(TA)]7TAA, is known to decrease the level of gene expression (30, 31). Variability in the expression of the UGT1A1 protein resulting from this polymorphism may lead to important differences in estrogen biotransformation and possibly explain interindividual differences in endometrial cancer risk. Our findings support a role for UGT1A1 in estrogen metabolism in the uterus and suggest that lower expression of UGT1A1 decreases the risk of endometrial cancer by altering the conjugation of 2-hydroxy estrogen metabolites.
MATERIALS AND METHODS

Glucuronidation Assay of Estrogens. The stable UGT1A1 protein was assessed with microsomal fractions incubated for 1 h in the presence of concentrations varying from 0.5 to 100 μM of E2, 2-OH-E2, and 4-OH-E2. Reactions (100-μl volume) contained 50 mM Tris-HCl (pH 7.5), 10 mM MgCl2, 100 μg/ml phosphatidylcholine, 8.5 mM sarcosyl, 2 mM UDP-glucuronic acid, 40–60 μg of membrane protein, and E2, 2-OH-E2, and 4-OH-E2 as substrates. Reactions were initiated by adding varying concentrations of each individual substrate. Blanks and controls contained all compounds except steroid substrates and microsomal preparation, respectively. The assays were terminated by adding 100 μl of methanol and centrifuged at 14,000 × g for 10 min. Reaction rates were linear during this period of time (1 h). Due to the lack of apparent enzyme latency, inclusion of detergent was found to be unnecessary for assessment of the full glucuronidating potential of UGT1A1-expressing HK293 cell membranes. Glucuronidation activities of UGT1A1 were expressed as pmol glucuronide/min/UGT1A1 protein level. The reported values for Vmax for the formation of estrogen glucuronides were observed experimentally. Values were expressed as the mean ± SD of a single experiment performed in triplicate. To ascertain the level of UGT1A1 protein expression in the stable UGT1A1-expressing HK293 cells, a semiquantitative immunoblot analysis method was used with an anti-UGT1A1 antibody as described previously (32–34).

Mass Spectrometry (MS) Analysis. The medium was diluted with 0.1 ml of methanol:water (50:50, v/v), vortexed, and then transferred into a conical vial for injection into the mass spectrometer. The high-performance liquid chromatography/Mass Spectrometry system consisted of a mass spectrometer Model API 300 (PE Scieix, Thornhill, Ontário, Canada) equipped with an electropray ionization (ESI) source in the negative ion mode and a high-performance liquid chromatography pump plus autosampler Model 2690 (Waters, Milford, MA). A chromatographic separation was achieved with a Xterra mass spectrometry (MS) C18 column 3.5 μm packing material, 100 × 3.9 mm (Waters, Milford, MA), using a three-solvent isocratic system (A, water + 0.1% ammonium hydroxide; B, methanol + 0.1% ammonium hydroxide; C, acetonitrile + 0.1% ammonium hydroxide) at a constant flow rate (0.8 ml/min), 8 min with 90% A, 8.5% B, and 1.5% C. Afterward, the column was washed with 93.5% B and then re-equilibrated to the initial condition over 4 min. The mass spectrometer operated in the multiple ion reaction monitoring mode: for estrogen glucuronide (E2G), parent ion m/z 447 and daughter ion m/z 113; for 2-OH-E2G, parent ion m/z 463 and daughter ion m/z 287; and for 4-OH-E2G, parent ion m/z 463 and daughter ion m/z 287. The quantification was performed by using a calibration curve in the range of 50–500 pg/ml for the products present in the medium for each substrate.

Expression Analysis of UGT1A1. Nonmalignant uterine tissue was obtained from postmenopausal women (n = 3) not taking hormone replacement therapy for at least 3 weeks and in whom menstrual bleeding stopped at least 1 year ago. All subjects provided written consent for the use of their specimens. The study was reviewed and approved by the Institutional Review Boards at CHUL Research Center, Hôtel-Dieu de Québec, and Laval University. Fresh materials from patients were collected from surgeries performed at the Hôtel-Dieu de Québec and prepared by the pathologist. Endometrial tissue was obtained by scraping from the posterior surface of the inner lining of the uterine fundus. Specimens were immediately deposited in liquid nitrogen within 30 min of surgery pending transfer to a freezer at −80°C. Total RNA was collected with Trizol (Molecular Research Center, Cincinnati, OH), and random hexamers (pDN6) were used to synthesize cDNA from total RNA (1 μg) using a SuperScript II cDNA synthesis kit (Invitrogen, Carlsbad, CA) according to the manufacturer’s specifications. Aliquots of the first-strand cDNA were used as templates for PCR amplification of UGT1A1 using Taq polymerase (PE Applied Biosystems, Branchburg, NJ). PCR was carried out using forward primer 5′-GAGGAGCTGACTCAGGAC-3′ and reverse primer 5′- CAAATTCCTGGGATAGTGGATTTT-3′ for UGT1A1, yielding a product of 155 bp, under the following conditions: 95°C for 3 min; followed by 35 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s; and a final extension time of 7 min at 72°C. With all reverse transcription-PCR reactions, a parallel aliquot of the same sample was run with reverse transcriptase omitted. The reverse transcription-PCR amplification products were analyzed by agarose gel electrophoresis and by direct sequencing with an ABI automated sequencer to confirm the identity of UGT1A1.

Western blot analyses were conducted to determine the level of UGT1A1 protein expressed in the microsomal fractions (20 μg) obtained from endometrial tissues using a previously described method (33). Polyclonal antibody specificity was confirmed by analyzing the reactivity against all known human UGT1 and UGT2 isoenzymes using microsomal fractions (10 μg) of UGT1A1-overexpressing HEK293 cells.

Study Population. The Nurses’ Health Study began in 1976 when 121,700 female United States registered nurses between the ages of 30 and 55 years completed a self-administered questionnaire on their medical histories and baseline health-related exposures. Information regarding endometrial cancer risk factors was obtained from biennial questionnaires and a questionnaire completed at the time of blood collection. These questionnaires include data on reproductive variables, oral contraceptive and postmenopausal hormone (PMH) use, cigarette smoking, and (since 1980) dietary intake. Between 1989 and 1990, blood samples were collected from 32,826 women. Approximately 97% of the samples were returned within 26 h of blood draw. On receipt, these samples were centrifuged immediately; aliquoted into plasma, RBCs, and buffy coat fractions; and stored in liquid nitrogen freezers. Women were defined as postmenopausal at the time of blood collection if they reported having a bilateral oophorectomy or no menstrual cycle within the last 12 months before blood draw. Menopausal status and PMH use were updated until the date of diagnosis for cases and matched controls. Subsequent follow-up has been >98% for this subcohort of Nurses’ Health Study participants who have given blood.

In our study, we included both incident and prevalent endometrial cancer cases from the Nurses’ Health Study blood cohort. Eligible incident cases consisted of women with pathologically confirmed invasive endometrial cancer that had been diagnosed any time after blood collection and up to June 1, 1998, with no previously diagnosed cancer except for nonmelanoma skin cancer. Prevalent cases were defined as having pathologically confirmed invasive endometrial cancer diagnosed between 1976 and the date of blood collection, with no previously diagnosed cancer except for nonmelanoma skin cancer. Controls for both incident and prevalent cases were randomly selected participants who had given a blood sample, had not had a hysterectomy, and were free of diagnosed cancer (except nonmelanoma skin cancer) up to and including the interval in which the case was diagnosed. Controls were matched to cases 3:1 according to year of birth, menopausal status at blood draw and at the cycle before diagnosis, and PMH use at time of blood draw (current users versus not current users). Controls were also matched to cases by time of day of blood collection, month of blood return, and fasting status at blood draw because of planned plasma analyses. This case-control study consists of 104 incident endometrial cancer cases, 118 prevalent endometrial cancer cases, and 1,236 control subjects. In addition, 324 women who were controls in a nested case-control study of breast cancer (35) and who had not had a hysterectomy and were free of cancer other than nonmelanoma skin cancer were also genotyped for the UGT1A1 polymorphism. The study protocol was approved by the Committee on Use of Human Subjects of the Brigham and Women’s Hospital (Boston, MA).

Molecular Analysis. DNA was extracted from buffy coat fractions using the Qiagen QIAamp Blood Kit (Qiagen, Inc., Chatsworth, CA). DNA samples from cases and controls were genotyped for the dinucleotide insertion/deletion present in the promoter region of the UGT1A1 gene using a previously described method (32). All genotyping was performed by laboratory personnel blinded to case-control status, and blinded quality control samples were inserted to validate genotyping identification procedures; concordance for blinded samples was 100%.

Statistical Analysis. We used a χ2 test to assess whether the UGT1A1 genotypes were in Hardy-Weinberg equilibrium and to determine P-values for differences in genotype frequencies between cases and controls. The association between the UGT1A1 genotypes and endometrial cancer risk were examined by using conditional and unconditional logistic regression to calculate odds ratios (ORs) and 95% confidence intervals (CIs). In addition to the matching factors, analyses were also adjusted for endometrial risk factors: body mass index [BMI (in kg/m2)] at age 18 years; weight gain since age 18 years (<5, 5–19.9, and ≥20 kg); age at menarche (<12, 12, 13, and ≥13 years); parity/age at first birth (nulliparous, ≥1 child/age at first birth ≤ 24 years, ≥1 child/age at first birth ≥ 24 years); pack-years of smoking (never

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RESULTS

Metabolic Activities of the UGT1A1 Protein and Its Expression in the Endometrium. Characterization of the kinetic parameters of estrogen glucuronidation activities for UGT1A1 toward E2, 2-OH-E2, and 4-OH-E2 was determined under the same experimental conditions using microsomal preparation of UGT1A1-overexpressing HEK293 cells. Before these experiments, the presence of the UGT1A1 protein in microsomes was confirmed by Western blot analysis as described previously (33). Under linear kinetic conditions, relative \( V_{\text{max}} \) values clearly indicated that UGT1A1 was most efficient in the formation of 2-OH-E2 compared with E2 and 4-OH-E2 (Fig. 1). UGT1A1 \( V_{\text{max}} \) for 2-OH-E2 was at least 11-fold greater for 2-OH-E2 compared with 4-OH-E2 and E2 (1073, 61, and 93 pmol/min/UGT1A1 protein level, respectively).

We further examined the expression of UGT1A1 in the endometrium by reverse transcription-PCR and Western blot analysis. UGT1A1 mRNA and protein were detected in the normal endometrium (Fig. 2). Although the sample size was small, the interindividual variability in the expression of UGT1A1 was evident.

UGT1A1 Polymorphism and Endometrial Cancer. Our study population included 222 endometrial cancer cases and 666 matched controls. The characteristics of cases and controls have been described previously (36). Briefly, endometrial cancer cases were more likely to be current PMH users, have a greater BMI before diagnosis, gained more weight between age 18 years and diagnosis, smoked fewer cigarettes, and have a family history of endometrial and colorectal cancer compared with controls.

The distribution of the UGT1A1 genotypes was compared between incident and prevalent endometrial cancer cases to determine whether the variant UGT1A1*28 allele was associated with survival. The genotype frequencies were similar between incident \( *1/*1 \), \( n = 57 \) (56%); \( *1/*28, n = 39 \) (39%); \( *28/*28, n = 5 \) (5%) and prevalent cases \( *1/*1, n = 58 \) (50%); \( *1/*28, n = 42 \) (42%); \( *28/*28, n = 10 \) (9%); \( \chi^2 = 1.65 \); degrees of freedom (df) = 2; \( P = 0.44 \). In addition, the number of deaths from endometrial cancer among the prevalent cases \( n = 2 \) (25% of all deaths among prevalent cases) was not different from the number of deaths observed among the incident cases \( n = 6 \) (50% of all deaths among incident cases) from endometrial cancer (\( P = 0.10 \)). Therefore, all incident and prevalent cases were combined for all statistical analyses.

Our analysis included 218 cases and 656 controls; 3 cases and 8 controls could not be genotyped for the UGT1A1 promoter polymorphism. In addition, women with the UGT1A1*33 allele \( [\text{TA}]_3, \text{TA} \); \( n = 3 \); one case and two controls] were excluded from the analysis. The UGT1A1*33 and the UGT1A1*34 \( [\text{TA}]_3, \text{TA} \) alleles, common in African-American populations (35, 37), were extremely rare or nonexistent in this case-control population. Most of the women in this study reported their ethnicity as Caucasian. The calculated frequencies of the UGT1A1*1 and UGT1A1*28 alleles were 66% and 34% in the controls, respectively, and 73% and 27% in the cases. These frequencies are similar to those previously observed in Caucasian populations (16, 35, 37). The distribution of the UGT1A1 genotypes was in Hardy-Weinberg equilibrium for the controls (\( \chi^2 = 3.08; \text{df} = 1; P = 0.08 \)) and for the cases (\( \chi^2 = 0.11; \text{df} = 1; P = 0.74 \)). In case-only analyses, the UGT1A1*28 allele was not associated with

smoker, <30 pack-years, and 30–50 pack-years); menopausal status at diagnosis (premenopausal, postmenopausal, and/or missing menopausal status); age at menopause (<48 years, 48 years ≤ age <50 years, 50 years ≤ age <55 years, ≥55 years); first-degree family history of endometrial cancer (yes/no); and first-degree family history of colorectal cancer (yes/no). Indicator variables for the UGT1A1 genotype were created using individuals homozygous for the UGT1A1*1 [A(TA)₆TAA] allele, UGT1A1*1/*1, as the reference category. Gene dosage effects were modeled by assigning the values of 0, 1, and 2 to a genotype trend variable according to the subject’s number of variant alleles (zero, one, and two variant alleles, respectively). If no gene dosage effect was observed, the genotype was also evaluated using a dichotomous variable (carrier versus noncarrier). Unconditional logistic regression models were used for all stratified analyses. To test statistical interactions between UGT1A1 genotypes and environmental exposures in the unconditional logistic regression models, we first used a likelihood-ratio test to compare nested models that included terms for all combinations of the genotypes and levels of environmental exposures to the models with indicator variables for the main effects only (nominal likelihood-ratio test). We also modeled UGT1A1 genotypes as ordinal variables and environmental exposures as continuous variables to assess the statistical significance of interactions by likelihood-ratio test of a single interaction term (ordinal likelihood-ratio test). Case-only analysis was conducted to investigate the association between the genotypes and the degree of differentiation of endometrial cancer using the \( \chi^2 \) test. All \( P \)-values are two-sided. We used the SAS (SAS Institute, Cary, NC) statistical package for all analyses (SAS, Version 8.2 for Unix).
any histopathological characteristics, including subtype and differentiation status.

There was a statistically significant difference in genotype frequencies for cases and controls ($\chi^2 = 8.79; \text{df} = 2; P = 0.01$). Compared with women homozygous for the $UGT1A1^{*1/1}$ allele, the adjusted ORs were 0.81 (95% CI, 0.56–1.16) for women with the $UGT1A1^{*1/28}$ genotype and 0.40 (95% CI, 0.21–0.75) for women with the $UGT1A1^{*28/28}$ genotype (Table 1). A statistically significant gene dosage effect of the $UGT1A1^{*28/28}$ allele and endometrial cancer risk was observed ($P_{\text{trend}} = 0.007$). Compared with the $UGT1A1^{*1/1}$ genotype, the adjusted OR for women carrying the $UGT1A1^{*28}$ allele was 0.71 (95% CI, 0.51–1.00).

Considering our finding, we sought to further define the $UGT1A1$ and endometrial cancer association by including a second population of control women ($n = 324$) from a nested breast cancer case-control study within the Nurses’ Health Study (35). A comparison of the population characteristics of the second control group with the first control group revealed no material differences in ethnicity and reproductive and hormone-related factors (data not shown). The calculated frequencies of the $UGT1A1^{*1/1}$ and $UGT1A1^{*28}$ alleles in this second control group were 68% and 32%; these frequencies were similar to those of the matched control group ($P > 0.05$). The distribution of the $UGT1A1$ genotypes was in Hardy-Weinberg equilibrium for the second population of controls ($\chi^2 = 2.34; \text{df} = 1; P = 0.13$). The association of the $UGT1A1$ polymorphism and endometrial cancer risk was fundamentally the same, with an adjusted OR of 0.72 (95% CI, 0.53–0.98).

We observed a potential interaction between $UGT1A1$ and menopausal status at diagnosis ($P$, test for nominal interaction = 0.05). Crude and fully adjusted ORs (Table 1) were similar; therefore, we did not adjust for the potential confounding risk factors to improve the precision of the stratum-specific effect estimates in this unconditional analysis. Among premenopausal women, the adjusted OR for women with a $UGT1A1^{*28}$ allele was 0.39 (95% CI, 0.18–0.85); for postmenopausal women, the adjusted OR for women with a $UGT1A1^{*28}$ allele was 0.79 (95% CI, 0.55–1.13) compared with women with the homozygous $UGT1A1^{*1/1}$ genotype.

Because $UGT1A1$ is involved in estrogen conjugation, BMI and PMH use are potential effect modifiers based on biological plausibility and their potential influence on estrogen levels. Although PMH use and increased BMI are well-established risk factors for endometrial cancer, no statistically significant interaction was observed with the $UGT1A1$ polymorphism and endometrial cancer risk. Compared with PMH never-users who were homozygous for the $UGT1A1^{*1}$ allele, the OR for women who were current users with the variant $UGT1A1^{*1/28}$ genotype was 4.95 (95% CI, 2.25–10.89), and the OR for women who were current PMH users with the variant $UGT1A1^{*28/28}$ allele was 2.24 (95%CI, 1.06–4.75; $P$, test for nominal interaction = 0.10). No significant association was observed regarding type of PMH use (estrogen versus estrogen + progestin), duration of PMH use, and BMI at diagnosis (data not shown); however, there was only limited power to detect such an association.

**DISCUSSION**

We initiated this study to understand the role of $UGT1A1$ in the metabolism of estrogens in the uterus and to determine the association between the common functional promoter variant and endometrial cancer risk. We characterized the $UGT1A1$ glucuronidation profiles for $E_2$, 2-OH-$E_2$, and 4-OH-$E_2$. Previous data have suggested that $UGT1A1$ was responsible for the conjugation of $E_2$ (18). Our data support these findings, and in addition we demonstrated that $UGT1A1$ has a preference for the glucuronidation of 2-OH-$E_2$. $UGT1A1$ was expressed in the nonmalignant endometrium, supporting its potential role in the local inactivation of 2-OH-$E_2$. Our nested case-control results suggest that the $UGT1A1^{*28/28}$ allele was inversely associated with endometrial cancer risk, indicating that the decreased expression of the $UGT1A1$ protein can potentially have a beneficial effect in the uterus.

Estrogen, along with progesterone, is an important determinant of cancer in the endometrium, and factors that alter serum hormonal levels influence endometrial cancer risk (12, 38). The bioavailability of steroids depends on their concentration in the systemic circulation, yet this is not the sole determinant of estrogen action in hormonal-

### Table 1: Associations between $UGT1A1$ genotype and endometrial cancer risk by menopausal status at diagnosis

<table>
<thead>
<tr>
<th>$UGT1A1$ genotype</th>
<th>Case (%)</th>
<th>Controls (%)</th>
<th>OR* (95% CI)*</th>
<th>OR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cases and controls&lt;sup&gt;1&lt;/sup&gt;</td>
<td>115 (52.6)</td>
<td>289 (44.1)</td>
<td>1.00 ref</td>
<td>1.00 ref</td>
</tr>
<tr>
<td>*1/1&lt;sup&gt;1&lt;/sup&gt;</td>
<td>88 (40.4)</td>
<td>279 (42.5)</td>
<td>0.79 (0.57–1.10)</td>
<td>0.81 (0.56–1.16)</td>
</tr>
<tr>
<td>*28/28&lt;sup&gt;1&lt;/sup&gt;</td>
<td>15 (6.9)</td>
<td>88 (13.4)</td>
<td>0.43 (0.24–0.78)</td>
<td>0.40 (0.21–0.75)</td>
</tr>
<tr>
<td>*1/28 + *28/28&lt;sup&gt;1&lt;/sup&gt;</td>
<td>103 (47.3)</td>
<td>367 (56.0)</td>
<td>$P_{\text{trend}} = 0.005$</td>
<td>0.71 (0.50–0.97)</td>
</tr>
<tr>
<td>Premenopausal women</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1/1&lt;sup&gt;1&lt;/sup&gt;</td>
<td>32 (59.3)</td>
<td>37 (39.8)</td>
<td>1.00 ref&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>*1/28&lt;sup&gt;1&lt;/sup&gt;</td>
<td>21 (38.9)</td>
<td>41 (44.1)</td>
<td>0.51 (0.22–1.14)&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>*28/28&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1 (1.9)</td>
<td>15 (16.1)</td>
<td>0.06 (0.01–0.54)&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>*1/28 + *28/28&lt;sup&gt;1&lt;/sup&gt;</td>
<td>22 (40.7)</td>
<td>56 (60.2)</td>
<td>0.39 (0.18–0.85)&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Postmenopausal women</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1/1&lt;sup&gt;1&lt;/sup&gt;</td>
<td>82 (51.3)</td>
<td>247 (45.1)</td>
<td>1.00 ref&lt;sup&gt;4&lt;/sup&gt;</td>
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</tr>
<tr>
<td>*1/28&lt;sup&gt;1&lt;/sup&gt;</td>
<td>64 (40.0)</td>
<td>230 (42.0)</td>
<td>0.85 (0.58–1.24)&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>*28/28&lt;sup&gt;1&lt;/sup&gt;</td>
<td>14 (8.8)</td>
<td>71 (13.0)</td>
<td>0.60 (0.32–1.12)&lt;sup&gt;4&lt;/sup&gt;</td>
<td>$P_{\text{trend}} = 0.11$</td>
</tr>
<tr>
<td>*1/28 + *28/28&lt;sup&gt;1&lt;/sup&gt;</td>
<td>78 (48.8)</td>
<td>301 (54.9)</td>
<td>0.79 (0.55–1.13)&lt;sup&gt;4&lt;/sup&gt;</td>
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<sup>1</sup> $P$ for interaction = 0.05
<sup>2</sup> OR, odds ratio; CI, confidence interval.
<sup>3</sup> Conditional logistic regression model adjusted for the matching variables (age, menopausal status at blood collection, postmenopausal hormone use at blood draw, date of blood draw, time of blood draw, and fasting status at blood draw).
<sup>4</sup> Conditional logistic regression adjusted for matching variables and body mass index at age 18 years, weight gain since age 18 years until diagnosis, age at menarche, age at menopause, postmenopausal hormone use at diagnosis, first-degree family history of uterine cancer and colorectal cancer, parity, age at first birth, and pack-years of smoking.
<sup>5</sup> "All cases and controls" included premenopausal and postmenopausal women and women with dubious menopausal status. Numbers may vary because of missing genotypes.
<sup>6</sup> Unconditional logistic regression adjusted for the matching variables (age, menopausal status at blood collection, postmenopausal hormone use at blood draw, date of blood draw, time of blood draw, and fasting status at blood draw).
<sup>7</sup> $P$ for the nominal likelihood-ratio test comparing the nested model that included terms for all combinations of the genotype and menopausal status at diagnosis to the model with indicator variables for the main effects only.
dependent tissues such as the uterus. Estrogen metabolism occurs via multiple pathways in target tissues, and the activity and expression profiles of these metabolic processes contribute significantly in the modulation of specific physiological effects of estrogens in target cells. Most biotransformation enzymes for E2 synthesis, conjugation, and inactivation are expressed in endometrial tissue (39–42). Interindividual variability in levels of conjugated estrogens, in the form of estrogen glucuronides, have been measured in urine, breast cyst fluid, and follicular fluid, suggesting that UGT enzymes have an effect on the inactivation and elimination of estrogens and their accumulation in target tissues (13, 32, 43, 44). Our previous work demonstrated that specific UGT enzymes help to maintain steady-state levels of steroids in target tissues (45).

The intracellular concentrations of estrogens are dependent on the interactions between multiple phase I and phase II enzymes. It is hypothesized that an increased formation of catechol estrogens mediated by cytochrome P450 enzymes coupled with insufficient glucuronidation of E2 and its metabolites may substantially increase the risk of endometrial cancer. It is well established that the coordinate hormonal milieu in estrogen and its metabolite concentrations are necessary to the function of the endometrium; endometrial carcinoma occurs as a result of imbalances between stimulatory factors such as estrogen and inhibitory factors such as progesterone and possibly protective estrogen metabolites in the endometrium. Based on the profile of expression and activity of the UGT1A1 enzyme, we hypothesized that the functional promoter polymorphism in the UGT1A1 gene may be important in estrogen metabolism by altering the conjugation of 2-OH-CE. A lower expression of UGT1A1 was predicted to lead to significantly elevated concentrations of 2-OH-CE and subsequent methylation to 2-MeO-CE in the endometrium and therefore a decreased risk of endometrial cancer. We observed an inverse association between the UGT1A1*28 allele and endometrial cancer risk, thus supporting our hypothesis. The 2-OH-CEs have the potential to undergo metabolic redox cycling and generate free radicals, but unlike 4-OH-CEs, 2-OH-CEs have no tumorigenic activity (6, 23) and possess weak hormonal potency in estrogen target tissues (47, 48). In addition, the metabolites of 2-OH-CEs, 2-MeO-CEs, are associated with antiproliferative, cytotoxic, and apoptotic activity (6, 23). Thus, a variation in local glucuronide conjugation of 2-OH-CE mediated by UGT1A1 could affect the level of such metabolites in the endometrium. Preliminary studies in our laboratory indicated that UGT1A1, in addition to the conjugation of 2-OH-CE, was also involved in the glucuronidation of 2-MeO-CE.10 As a consequence, reduced expression would result not only in higher levels of 2-OH-CE, the precursor of 2-MeO-CE, but also in increased concentrations of 2-MeO-CE in target cells.

We observed a potential interaction between UGT1A1 and menopausal status at diagnosis. The association between the UGT1A1 genotype and endometrial cancer was stronger among premenopausal women compared with postmenopausal women, consistent with a hormonal-based mechanism whereby the production and fluctuation in E2 levels are more important before menopause.

We have previously evaluated the low transcription UGT1A1 promoter allele, UGT1A1*28, with breast cancer risk in a nested case-control study within the Nurses’ Health Study and found no significant association with breast cancer risk or plasma hormone levels (35). In a second population-based, case-control study, we observed a 2-fold increased risk of breast cancer in premenopausal African-American women with seven and eight TA repeats compared with women with five and six TA repeats (32). Although this finding needs to be replicated, the initial findings would indicate that the UGT1A1 promoter genotype has differential effects on breast and endometrial cancer risk. Differences in metabolic routes between these two target tissues have previously been shown to influence the action of estrogen metabolites in a tissue-specific manner (3, 49). This is likely, considering that estrogen metabolites have tissue-specific biological activities, most likely due to their local metabolism (3, 50, 51).

In summary, our results suggest that inherited alterations in the expression of the UGT1A1 gene result in interindividual differences in estrogen metabolism that ultimately may result in decreased endometrial cancer risk. Our findings suggest that less glucuronidation of 2-OH-CE protects against endometrial carcinogenesis and that UGT1A1 is a key player in this process. This is the first nested case-control study to observe an association between the phase II enzyme UGT1A1 and endometrial cancer risk. Larger studies are warranted to confirm the inverse association observed between the UGT1A1 functional dinucleotide repeat polymorphism and endometrial cancer risk and to further examine interactions between UGT1A1 and endometrial cancer risk factors.

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REFERENCES


10 C. Guillemette, unpublished observations.


The Functional $UGT1A1$ Promoter Polymorphism Decreases Endometrial Cancer Risk

Yannick Duguay, Monica McGrath, Johanie Lépine, et al.

Cancer Res 2004;64:1202-1207.

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