Genetic Polymorphisms in Uridine Diphospho-Glucuronosyltransferase 1A1 and Association with Breast Cancer among African Americans

Chantal Guillemette, Robert C. Millikan, Beth Newman, and David E. Housman

ABSTRACT

We examined the role of constitutional genetic variation at the UDP-glucuronosyltransferase (UGT) 1A1 locus in breast cancer susceptibility. The UGT1A1 enzyme is a major UGT involved in estradiol glucuronidation. To date, four UGT1A1 variant alleles characterized by a variation in the number of TA from five through eight repeats in the atypical TATA box region have been described in the African-American population. Functional analyses of the transcriptional activity in breast and liver cells revealed that the transcription activation of a reporter gene is inversely correlated with the number of repeats. Reverse transcription-PCR analysis confirmed the expression of UGT1A1 in human liver in the hepatocarcinoma cell line HepG2 and provided evidence of the expression of UGT1A1 in breast cancer tissue, where a positive signal was observed in 11 of 12 breast cancer cell lines tested. The population-based case-control study involved 200 women with breast cancer and 200 female controls of African ancestry. We postulated that breast cancer cases might have a higher prevalence of low activity allele-containing genotypes than controls (alleles presenting seven and eight repeats in the A(TA)nTAA motif of the TATA box). The age-adjusted odds ratio (OR) for breast cancer comparing women with seven and eight allele-containing genotypes versus 5/5, 5/6, and 6/6 genotypes was 1.8 (95% confidence interval (CI), 1.0–3.1; \( P = 0.06 \)) in premenopausal women and 1.0 (95% CI, 0.5–1.7; \( P = 0.9 \)) in postmenopausal women. The observed 1.8-fold elevated risk in premenopausal women with invasive breast cancer is highly suggestive of a possible interaction between UGT genotype and hormones. Additional analyses suggested a stronger association of UGT1A1 genotype with estrogen receptor (ER)–negative breast cancer. Among premenopausal women, the association was stronger for ER– breast cancer (OR, 2.1; 95% CI, 1.0–4.2; \( P = 0.04 \)) than ER+ breast cancer (OR, 1.3; 95% CI, 0.6–3.0; \( P = 0.5 \)). The OR was slightly stronger among women who used oral contraceptives, and the association remained null in postmenopausal women, regardless of whether they took hormone replacement therapy. Our current findings suggest that further investigations are warranted to elucidate the role of UGT1A1 in breast cancer risk.

INTRODUCTION

Inherited variations in genes involved in the metabolism of estrogens, in addition to those of carcinogens, are suggested to be associated with an increased risk of breast cancer. This hypothesis was tested in a number of epidemiological studies that have focused on polymorphisms present in different enzymatic pathways including cytochrome P450 enzymes (1–5), catechol-O-methyltransferase (6, 7), glutathione S-transferases (8, 9), and N-acetyltransferases (10–12).

One putative susceptibility locus that has not yet been explored is the UGT locus encoding the UGTS. UGTS catalyze the glucuronidation reaction, which represents a major pathway in phase II drug metabolism (13). They play a major role in the detoxification of a diverse range of molecules, including carcinogens and biologically active endogenous compounds, such as steroid hormones. An additional role of UGT enzymes is to maintain intracellular steady-state levels of steroids, including estrogens, in target tissues (14). High levels of estrogen glucuronides have been observed in breast cyst fluid, suggesting their formation within the mammary gland (15, 16). Prospective studies have shown that breast cancer cases present higher serum levels of estrogens, namely estradiol and estrone, compared to controls (17, 18). Changes in estrogen metabolism, suggested as a biomarker for breast cancer, may be caused to some extent by an alteration in the glucuronidation pathway, which directly inactivates estrogens and facilitates their elimination from their site of action. To date, several UGTs have shown glucuronidation activity for estrogens and their metabolites, catechol estrogens (19–21). Additional studies suggest the major contribution of a specific member of the UGT1 family, UGT1A1, in estradiol-glucuronide formation, with the observation that Crigler-Najjar patients, deficient in UGT1A1, present a 70% decrease in the glucuronidation of estradiol compared to normal individuals (20). The more common genetic variant described to date in the UGT1A1 gene is a dinucleotide repeat polymorphism in the atypical TATA box region of the UGT1A1 promoter. The variant allele consists of seven TA repeats in the A(TA)nTAA motif, whereas six TA repeats characterize the common allele (UGT1A1*1). The presence of the (A(TA))7 TAA allele (UGT1A1*28) was previously found to decrease UGT1AI gene expression in vitro. Homozygous individuals carrying the A(TA)7 TAA allele (1–19% of the population) show significantly higher plasma levels of unconjugated bilirubin caused by a 30% reduction in UGT1AI gene transcription (22–25).

In the present study, we first investigated the expression of UGT1A1 in human breast cancer cell lines. We subsequently evaluated the transcriptional activity of the different UGT1A1 promoters found in the human population, including two additional UGT1A1 alleles also characterized by a variation in the number of TA repeats in the promoter region that have been recently reported in an African-American population (25). Next, we investigated the association between genetic variability in the UGT1A1 promoter region and risk of breast cancer in a population-based case-control study of African-American women. The relationship between the glucuronidation pathway and breast cancer has never been explored in epidemiological studies; this study constitutes the first report of genetic polymorphism in UGT enzymes and susceptibility to cancer.

MATERIALS AND METHODS

Expression Analysis. Cell lines were obtained from the American Type Tissue Collection (Manassas, VA). Total RNA was collected from the cells with Trizol (Life Technologies, Inc., Grand Island, NY) following the manufacturer’s instructions. Oligo(dT) primer cDNA was synthesized from total RNA (5 µg) using a SuperScript II cDNA synthesis kit (Life Technologies, Inc., Grand Island, NY). cDNA was amplified 25 cycles, and 2 µl of each PCR product was separated on a 2% agarose gel.
Inc.) according to the manufacturer’s specifications. Aliquots of the first-strand cDNA were used as templates for PCR amplification of UGT1A1 and GAPDH transcripts using Taq DNA polymerase (PE Applied Biosystems, Branchburg, NJ). PCR was carried out using forward primer 5'-AACAAAGGACCTACGGCCCTCC-3' and reverse primer 5'-GGTCCGAAATTCGTGCTGG-3' for UGT1A1 and forward primer 5'-TGGTTGGAAACCATGAGG-3' and reverse primer 5'-CCAGGCTCAAAAGTTGG-3' for GAPDH under the following conditions: (a) 94°C for 5 min; (b) 30 cycles of 94°C for 1 min, 59°C for 1 min, and 72°C for 1 min and 30 s; and (c) a final extension time of 10 min at 72°C. The RT-PCR amplification products were analyzed by agarose gel electrophoresis, and direct sequencing was performed with an ABI automated sequencer to confirm the identity of UGT1A1. The UGT1A1 primers recognize sequences in the first exon of the UGT1A1 and give a product of 644 bp (26).

With all RT-PCR reactions, a parallel aliquot of the same sample was run in which reverse transcriptase was omitted. In these samples, no bands were seen, indicating the absence of contaminating DNA or of PCR carryover.

Promoter Activity. For transcriptional assays, we used the pGL3-Luc reporter plasmids (Promega Corp., Madison, WI). The 5'-flanking region of UGT1A1 was amplified by PCR using germ-line DNA from individuals genotyped 5/5, 6/6, 7/7, and 7/8 and the 5' primer 5'-CCGAGCTCGAGGTTGCTGGAATGTCCCTG-3' and the 3' primer 5'-CCGCCTGAGCGGCGCTTGTGCTCCTGCAAGA-3. The 5' end of the amplified fragment contained nucleotide −208, and the 3' end stopped at nucleotide −1. The PCR product was subcloned into pGL3 after digestion with SalI-XhoI, restriction sites introduced with the PCR primers. PCR amplification was performed using Pfu turbo DNA polymerase (Stratagene, La Jolla, CA) in a 50-μl reaction. The number of repeats and the absence of point mutation were confirmed by sequencing. Constructs characterized by four and nine TA repeats in the promoter region of UGT1A1, as described below, were introduced with the PCR primers. PCR amplification was performed using Pfu Turbo DNA polymerase (PE Applied Biosystems, Branchburg, NJ). PCR was carried out using forward primer 5'-AACAAAGGAGCTACGGCCCTCC-3' and reverse primer 5'-GGTCCGAAATTCGTGCTGG-3' for UGT1A1 and forward primer 5'-TGGTTGGAAACCATGAGG-3' and reverse primer 5'-CCAGGCTCAAAAGTTGG-3' for GAPDH under the following conditions: (a) 94°C for 5 min; (b) 30 cycles of 94°C for 1 min, 59°C for 1 min, and 72°C for 1 min and 30 s; and (c) a final extension time of 10 min at 72°C. The RT-PCR amplification products were analyzed by agarose gel electrophoresis, and direct sequencing was performed with an ABI automated sequencer to confirm the identity of UGT1A1. The UGT1A1 primers recognize sequences in the first exon of the UGT1A1 and give a product of 644 bp (26).

With all RT-PCR reactions, a parallel aliquot of the same sample was run in which reverse transcriptase was omitted. In these samples, no bands were seen, indicating the absence of contaminating DNA or of PCR carryover.

Population. The CBCS is a population-based, case-control study of breast cancer in North Carolina (27). Women with a first diagnosis of histologically confirmed, invasive breast cancer were identified through a rapid ascertainment system with the help of the North Carolina Central Cancer Registry (28). Controls were selected from lists provided by the North Carolina Division of Motor Vehicles (women ages 20–64 years) and the United States Health Care Financing Administration (women ages 65–74 years). Randomized recruitment (29) was used to select approximately equal numbers of African-American and white women, as well as equal numbers of women younger than age 50 and age 50 or older, among cases and controls. Controls were frequency-matched to cases by race and by 5-year age group. Between May 1993 and December 1996, 886 cases and 841 controls were enrolled. Overall response rates were 74% among cases and 53% among controls. Response rates were lower among younger African-American controls and older African-American cases (30). Interviews were conducted in participants’ homes by trained nurse-interviewers, who collected information on participants’ reproductive history, diet and lifestyle factors, a detailed family history of cancer, and occupational history. Race was classified according to self-report. Approximately 98% of participants who were interviewed agreed to give a 30-ml blood sample at the time of interview. Informed consent to obtain DNA was sought using a form approved by the Institutional Review Board of the University of North Carolina School of Medicine. For the present analysis of UGT1A1 genotypes, 200 African-American cases and 200 African-American controls were selected at random from the entire data set of 335 African-American cases and 332 African-American controls.

UGT1A1 Promoter Genotyping. Germ-line DNA was extracted from peripheral blood leukocytes according to standard methods (31). A total of 400 ng of genomic DNA were subjected to PCR analysis in 50-μl aliquots containing 20 pmol of each primer, 1× reaction buffer [50 mM KCl, 1.5 mM MgCl₂, and 10 mM Tris (pH 8.5)], 100 μM deoxynucleotide triphosphates, 4% DMSO, and 2 units of Taq DNA polymerase (PE Applied Biosystems). Ten sets of primers (listed in Table 1) were used to amplify the genomic region containing the TA repeats. In each primer set, one primer is fluorescence-tagged with FAM, TET, or HEX. The same PCR conditions were used for all primers, using the hot start technique (32) under the following conditions: 5 cycles of 30 s at 94°C, 45 s at 60°C, and 60 s at 72°C; followed by 30 cycles of 30 s at 94°C, 45 s at 55°C,
and 60 s at 72°C. An initial denaturation at 94°C for 5 min and a final extension at 72°C for 10 min were used. To verify the successful amplification of the promoter region of UGT1A1, 10 μl of PCR products were analyzed using agarose gel electrophoresis. Small aliquots (2 μl) of the amplified fluorescent PCR products from all 10 sets of primers were then mixed in a total volume of 20 μl with the molecular weight markers fluorescence tagged (carboxytetramethylrhodamine). One μl of each pool containing all 10 PCR products was then separated on a fragment analysis gel on the ABI Prism 377 DNA Sequencer and analyzed by GENESCAN2.1 Analysis software (PE Applied Biosystems). The fluorescence color and the variance of PCR length from 162–376 bp make it possible to pool PCR products for analysis on a polyacrylamide gel (Fig. 1B). The accuracy of the genotyping method was verified by sequencing of randomly selected PCR products.

Statistical Analysis. Student’s t test was performed to compare the level of promoter activity to that of the wild-type allele [−106 t; A(TA)6_TAA]. ORs and 95% CIs for breast cancer were calculated from unconditional logistic regression models and used to estimate relative risk (33). We controlled for age as an 11-level ordinal variable that reflected 5-year age categories, as well as additional covariates, using the SAS software package (version 6.11; SAS Institute, Cary, NC).

RESULTS

To ascertain the expression of UGT1A1, we performed RT-PCR analysis on a human liver sample, 12 breast cancer cell lines, and cell lines derived from metabolizing tissues including liver (HepG2), colon (Caco-320), and kidney (786-O). Eleven of the 12 breast cancer cell lines have shown the presence of a single RT-PCR product corresponding to UGT1A1 (Fig. 2). Specific amplification of UGT1A1 was also detected in human liver and in the hepatocarcinoma cell line HepG2. Additional cell lines derived from the colon and the kidney also expressed the UGT1A1 transcript. All cell lines tested and the human liver sample (including breast cancer cell line CAMA1, which does not present UGT1A1-positive amplification) were amplified for GAPDH.

Functional studies were subsequently carried out to confirm the ability of the different UGT1A1 promoter containing four to nine repeats to promote the expression of a luciferase reporter gene in breast cancer cells compared to liver cells. The alleles UGT1A1*1 [A(TA)6_TAA] and UGT1A1*28 [A(TA)8_TAA] have previously been reported in Caucasian population by several groups, whereas, the A(TA)nTAA and A(TA)nTAA alleles have been found exclusively in the African-American population (25). The two newly discovered UGT1A1 alleles, A(TA)nTAA and A(TA)nTAA, are referred to as UGT1A1*33 and UGT1A1*34 (Fig. 1C). To determine the functional significance of the TA insertion/deletion, 208-bp fragments representing all variant alleles were isolated by PCR using proofreading polymerase, cloned into the pGL3 reporter vector transfected in mammalian cells, and assessed for firefly luciferase reporter gene expression. All constructs were transfected into the human hepatocarcinoma cell line HepG2 and two breast cancer cell lines distinct with respect to ER status, namely, MCF7 (ER−) and MDA MB 468 (ER−). Results indicated that the variation in the number of TAs at this polymorphic locus differentially regulates transcription. The transcriptional activation of the luciferase reporter gene was decreased by 50% and 30% and increased by 20% using the allele A(TA)nTAA, A(TA)nTAA, and A(TA)nTAA constructs, respectively (Fig. 3, B and C). The relative level of transcription was 3.3-fold lower in MCF7 compared to the liver cell line and 5-fold higher in ER+ compared to ER− breast cancer cells. The number of repeats was shown to affect the transcription of UGT1A1 with the same magnitude in all cells tested (Fig. 3A). Further analysis of the sequence of the UGT1A1 promoter revealed another nucleic acid change at position −106 relative to the transcription initiation site characterized by the transition of a thymine to a cytosine, in linkage with the allele presenting five TA repeats. Because this nucleic acid change near the TATA box may affect the fully assembled transcriptional apparatus and the efficient interaction between the several components of the transcription machinery, we produced the mutation (T→C) at position −106 in the wild-type allele and converted the C to a T in the mutant allele.

Table 1 UGT1A1 PCR primers used for the genotype analysis

<table>
<thead>
<tr>
<th>PCR product</th>
<th>Fluorescent dye</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5′FAM</td>
<td>*AAG TGA ACT CCC TGC TAC CTT T</td>
<td>GCC GCC TTT GCT CCT GCC AGA</td>
</tr>
<tr>
<td>2</td>
<td>5′FAM</td>
<td>*AAG TGA ACT CCC TGC TAC CTT T</td>
<td>CCA CTG GGA TCA ACA GTA CT</td>
</tr>
<tr>
<td>3</td>
<td>5′FAM</td>
<td>*AAG TGA ACT CCC TGC TAC CTT T</td>
<td>CCA AGC ATG TCT AGC CAG</td>
</tr>
<tr>
<td>4</td>
<td>5′TET</td>
<td>*GAG GTT CGT GAA GTA CTT TGC C</td>
<td>GGC GCC TTT GCT CCT GCC AGA</td>
</tr>
<tr>
<td>5</td>
<td>5′TET</td>
<td>*GAG GTT CGT GAA GTA CTT TGC C</td>
<td>TTT GCT CTC GCC AGC AGT GGT</td>
</tr>
<tr>
<td>6</td>
<td>5′TET</td>
<td>*GAG GTT CGT GAA GTA CTT TGC C</td>
<td>CCA CTG GGA TCA ACA GTA CT</td>
</tr>
<tr>
<td>7</td>
<td>5′HEX</td>
<td>*AAG TGA ACT CCC TGC TAC CTT T</td>
<td>GCC GCC TTT GCT CCT GCC AGA</td>
</tr>
<tr>
<td>8</td>
<td>5′HEX</td>
<td>GTC AGC TGA AAC AGT CAA AC</td>
<td>*GTA CAA CCA GGC GTC AGG TGC</td>
</tr>
<tr>
<td>9</td>
<td>5′HEX</td>
<td>GTA CCA TGG G</td>
<td>*GTA CAA CCA GGC GTC AGG TGC</td>
</tr>
<tr>
<td>10</td>
<td>5′HEX</td>
<td>AAG TGA ACT CCC TGC TAC CTT T</td>
<td>*GTA CAA CCA GGC GTC AGG TGC</td>
</tr>
</tbody>
</table>

Fig. 2. Ethidium bromide-stained agarose gel of UGT1A1 and GAPDH RT-PCR products.
with five repeats. No dramatic effects were observed in HepG2, MCF7, or MDA MB 468 cells (Fig. 3, B and C).

To address the association between UGT1A1 genotype and breast cancer, we used a population of 200 breast cancer cases and 200 matched controls of African ancestry. We focused on African-American women in this study because all four UGT1A1 promoter alleles are present in this population. Characteristics of the samples genotyped are listed in Table 2. Participants who were genotyped did not differ substantially from African-American participants who were not genotyped for the risk factors listed above. These risk factors were similar among genotyped participants compared to the data set as a whole. We compared the distribution by stage among participants who were genotyped to that among participants who were not genotyped.

The main effect for UGT1A1 genotypes was estimated using ORs. We conducted our analysis by dividing participants based on the functional studies, where A(TA)5 TAA and A(TA)6 TAA represent high activity alleles, and A(TA)7 TAA and A(TA)8 TAA represent low activity alleles. All comparisons were achieved with the reference group including five or six allele-containing genotypes (5/5, 5/6, and 6/6; Table 4). The hypothesis was that low UGT1A1 activity genotypes would be associated with an increased susceptibility to estrogen-related cancers. Therefore, we postulated that breast cancer cases would have a higher prevalence of seven or eight allele-containing, high-risk genotypes (6/7, 6/8, 7/7, 7/8, 6/7, 5/7, and 5/8) than controls.

On stratification by menopausal status, association between UGT1A1 high-risk genotypes and risk of breast cancer showed an OR of 1.8 in those reported previously by Beutler et al. (25), with frequencies of 0.085 and 0.025 for alleles A(TA)5 TAA and A(TA)6 TAA, respectively, and frequencies of 0.49 and 0.40 for the most common alleles, A(TA)5 TAA and A(TA)6 TAA, respectively. To assess departures from Hardy-Weinberg equilibrium, we compared the observed genotype frequencies with the expected genotype frequencies (calculated on the basis of observed allele frequencies) among cases and controls using a \( \chi^2 \) test (34). Differences were not statistically significant among cases (\( \chi^2 \) test, \( P = 0.9 \)) or controls (\( \chi^2 \) test, \( P = 0.9 \)).

The main effect for UGT1A1 genotypes was estimated using ORs. We conducted our analysis by dividing participants based on the functional studies, where A(TA)5 TAA and A(TA)6 TAA represent high activity alleles, and A(TA)7 TAA and A(TA)8 TAA represent low activity alleles. All comparisons were achieved with the reference group including five or six allele-containing genotypes (5/5, 5/6, and 6/6; Table 4). The hypothesis was that low UGT1A1 activity genotypes would be associated with an increased susceptibility to estrogen-related cancers. Therefore, we postulated that breast cancer cases would have a higher prevalence of seven or eight allele-containing, high-risk genotypes (6/7, 6/8, 7/7, 7/8, 6/7, 5/7, and 5/8) than controls. On stratification by menopausal status, association between UGT1A1 high-risk genotypes and risk of breast cancer showed an OR of 1.8 in
premenopausal women (95% CI, 1.0–3.1; \( P = 0.06 \)) and an OR of 1.0 in postmenopausal women (95% CI, 0.5–1.7; \( P = 0.9 \); Table 4). Adjusted ORs were also higher among premenopausal women than among postmenopausal women when we used only 7/7 and 7/8 genotypes as the index group: OR = 2.0 (95% CI, 0.9–4.3; \( P = 0.08 \)) and OR = 1.0 (95% CI, 0.4–2.1; \( P = 0.9 \)), respectively. ORs did not differ substantially after controlling for the traditional breast cancer risk factors listed in Table 2.

To more fully address the possibility that UGT genotype may be related to hormone-dependent breast cancer, we calculated the ORs for ER+ breast cancer versus controls and for ER− breast cancer versus controls. Among all women, age-adjusted ORs for UGT1A1 seven and eight-allele-containing genotypes versus 5/5, 5/6, and 6/6 were 1.0 (95% CI, 0.6–1.7; \( P = 0.9 \)) for ER+ breast cancer and 1.5 (95% CI, 0.8–2.5; \( P = 0.2 \)) for ER− breast cancer. Among premenopausal women, the corresponding ORs were 1.3 (95% CI, 0.6–3.0; \( P = 0.5 \)) and 2.1 (95% CI, 1.0–4.2; \( P = 0.04 \)). ORs were close to the null value for ER+ and ER− breast cancer among postmenopausal women (Table 4).

We also assessed modification of ORs for the UGT1A1 genotype according to the use of exogenous hormones. Age-adjusted ORs for UGT1A1 seven and eight allele-containing genotypes versus 5/5, 5/6, and 6/6 were 1.5 (95% CI, 0.9–2.5; \( P = 0.2 \)) for women who ever used oral contraceptives and 1.1 (95% CI, 0.6–2.1; \( P = 0.8 \)) among never users. Among postmenopausal women, ORs were 1.0 (95% CI, 0.4–2.8; \( P = 0.9 \)) among ever users of hormone replacement therapy and 0.9 (95% CI, 0.4–1.8; \( P = 0.7 \)) among never users.

### DISCUSSION

In humans, high levels of conjugate estrogens in the form of glucuronide (G) are detected in the systemic circulation, urine, breast cyst fluid, and follicular fluid. Formation of steroids-G, including estrogens-G, by human mammary cancer cells, breast tumors, and metastasis lymph nodes has been demonstrated previously (15, 16, 35–40). The UGT1A1 isoenzyme has been specifically shown to efficiently conjugate estradiol in vitro (20), and confirmation of the ability of UGT1A1 to catalyze the glucuronidation of estradiol was ascertained in the present study using a HEK-293-UGT1A1 stable cells line (by C. G., data not shown). In vivo data reporting a significantly lower ability to glucuronidate estradiol in a UGT1A1-deficient patient also support the major contribution of UGT1A1 in estradiol-glucuronide formation in humans.

Until recently, UGT enzymes were believed to be expressed mainly in the liver. However, extrahepatic tissues present a wide expression of UGTs, and certain UGTs are in fact specifically expressed in tissues other than the liver (14, 41, 42). Although UGT1A1 mRNA has previously been identified in extrahepatic tissues, to date, the presence of this specific UGT has not been demonstrated in breast tissue. Our first experiment was intended to determine the expression of UGT1A1 at the site of action of estrogen. The data presented in this study provide evidence for the expression of UGT1A1 in numerous breast cancer cell lines and reinforce the notion that UGT1A1 is a major enzyme involved in estrogen-glucuronide formation in breast tissue.

The results of our functional studies confirmed the previous findings of Beutler et al. (25) that increasing the number of repeats in the promoter region leads to a decrease in the rate of transcription initiation of the UGT1A1 gene and identify UGT1A1 expression in breast cancer cells. Lower expression of UGT1A1 might lead to an increase in the level of estradiol, and expose cells to a higher local concentration of active hormone and therefore have considerable impact on tumor initiation and growth. Accordingly, UGT is possibly one of many factors determining individual estrogen exposure over a long time period. Unlike mutation in high penetrance genes such as BRCA1 and BRCA2, genetic variations in UGT1A1 are common among the general population and can therefore result in a large fraction of population risk. No studies to date have examined the genetic variation in UGT genes and a possible association with cancer.

In this pilot study of 200 cases and 200 controls, we had approx-

---

Table 3: Allele frequencies and genotype frequencies for UGT1A1 promoter in the SCBCs

<table>
<thead>
<tr>
<th>A. Allele(^a)</th>
<th>Cases(^b)</th>
<th>Controls(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.09</td>
<td>0.08</td>
</tr>
<tr>
<td>6</td>
<td>0.46</td>
<td>0.52</td>
</tr>
<tr>
<td>7</td>
<td>0.42</td>
<td>0.38</td>
</tr>
<tr>
<td>8</td>
<td>0.03</td>
<td>0.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Genotype(^c)</th>
<th>Cases (n (%))(^d)</th>
<th>Controls (n (%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>5/5</td>
<td>2 (1.0)</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>5/6</td>
<td>17 (8.5)</td>
<td>19 (9.5)</td>
</tr>
<tr>
<td>5/7</td>
<td>14 (7.0)</td>
<td>10 (5.0)</td>
</tr>
<tr>
<td>5/8</td>
<td>2 (1.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>6/6</td>
<td>46 (23.0)</td>
<td>56 (28.0)</td>
</tr>
<tr>
<td>6/7</td>
<td>73 (36.5)</td>
<td>72 (36.0)</td>
</tr>
<tr>
<td>6/8</td>
<td>3 (1.5)</td>
<td>4 (2.0)</td>
</tr>
<tr>
<td>7/7</td>
<td>38 (19.0)</td>
<td>33 (16.5)</td>
</tr>
<tr>
<td>7/8</td>
<td>5 (2.5)</td>
<td>5 (2.5)</td>
</tr>
<tr>
<td>8/8</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>200</td>
</tr>
</tbody>
</table>

\(^a\) \(X^2\) test comparing distribution in cases versus controls: \( P = 0.8 \)

\(^b\) Number of alleles/number of chromosomes (unweighted).

\(^c\) \(X^2\) test comparing distribution in cases versus controls: \( P = 0.8 \).

\(^d\) Number of participants with genotype/total participants (unweighted).
It is more likely that an individual carrier of low activity UGT1A1 alleles would present higher physiological concentrations of estradiol. The proliferative pressure imposed by the higher hormone levels throughout life in women presenting low UGT1A1 activity alleles, combined with other possible genetic predispositions and carcinogen exposure, can certainly contribute to the occurrence of early-onset breast cancer. In the present study, we observed the low activity alleles to be strongly associated with invasive breast cancer in women of African ancestry. A positive association for the UGT1A1 genotype was observed among premenopausal women (OR, 1.8; 95% CI, 1.0–3.1; \( P = 0.06 \)), which was stronger for ER- breast cancer (OR, 2.1; 95% CI, 1.0–4.2; \( P = 0.04 \)). These results are consistent with a role for UGT1A1 in modulating the effect of endogenous hormones on breast cancer risk.

In conclusion, based on the function of UGT1A1 in estrogen metabolism, it is more likely that an alteration in the level of transcription of this gene will not only have a profound impact on the inactivation of estrogens but also on their accumulation in the target cell, as well as preventing further activation by other steroid-transforming enzymes such as cytochrome P450 enzymes. The pattern of results suggests a stronger association of the UGT1A1 genotype with premenopausal breast cancer and ER- breast cancer. No significant interactions were observed with oral contraceptive use and hormone replacement therapy. To further explore cancer-susceptibility association, we suggest the analysis of the phenotype-genotype with larger numbers of women, including Caucasian women, and data on plasma hormone levels and breast cancer. Therefore, it is expected that a combination of the increased formation of catechol estrogens mediated by the cytochrome P450 enzymes added to an insufficient conjugation of the estradiol and estrogen metabolites via glucuronidation might greatly increase the risk of breast cancer. On the other hand, competing conjugative pathways significantly involved in the metabolism of estrogens, such as the sulfation, need to be considered as a possible mechanism that can compensate for the deficient glucuronidation of estrogens and may explain the marginally significant effect observed on breast cancer risk.

ACKNOWLEDGMENTS

We gratefully thank Dr. Anil G. Menon (University of Cincinnati, Cincinnati, OH) for providing additional germ-line DNAs from unrelated individuals of African ancestry. We also thank C-K. Tse for expert assistance in statistical programming and Dr. J. Borrow for critical reading of the manuscript.

REFERENCES


Genetic Polymorphisms in Uridine Diphospho-Glucuronosyltransferase 1A1 and Association with Breast Cancer among African Americans


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/60/4/950

Cited articles
This article cites 46 articles, 23 of which you can access for free at:
http://cancerres.aacrjournals.org/content/60/4/950.full.html#ref-list-1

Citing articles
This article has been cited by 28 HighWire-hosted articles. Access the articles at:
/content/60/4/950.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.