Association of Genetic Polymorphisms in UGT1A1 with Breast Cancer and Plasma Hormone Levels

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Abstract

UDP-glucuronosyltransferases (UGTs) catalyze the detoxification and the elimination of a large number of endogenous and exogenous compounds in the liver and extrahepatic tissues. One of the UGT1A family members, UGT1A1, is involved in estradiol metabolism and, therefore, represents a candidate gene in breast carcinogenesis. A common insertion/deletion polymorphism in the TATA-box of the promoter region of UGT1A1 results in decreased initiation of transcription. In a previous study, we found a positive association between the UGT1A1 low-transcriptional alleles and premenopausal breast cancer risk in an African-American population. In the present study, we sought to determine whether the low-transcription UGT1A1 promoter allele, UGT1A1*28 [A(TA)7 TAA], was associated with increased breast cancer risk among primarily Caucasian women in a nested case-control study within the Nurses’ Health Study cohort. No significant association between the UGT1A1*28 [A(TA)7 TAA] allele and breast cancer was observed. Compared with women homozygous for the UGT1A1*1 [A(TA)5 TAA] allele, the relative risk was 0.80 (confidence interval 0.49–1.29) for women homozygous for the UGT1A1*28 allele. The effect of the UGT1A1 genotype on plasma hormone levels in postmenopausal women not using hormone replacement was also evaluated, and overall, no significant differences in hormone levels by genotypes were observed. When restricted to women who had at least one UGT1A1*28 allele and a body mass index at blood draw of >27 kg/m2, particularly in combination with the cytochrome p450c17α genotype, estrone and estradiol levels tended to vary by UGT1A1 genotypes. The results presented do not support a strong association between the UGT1A1 promoter polymorphism and the risk of breast cancer.

Introduction

UGT1A1 represents one of the most important classes of phase II detoxification enzymes that catalyze the transfer of glucuronic acid to a large number of substrates in the liver and extrahepatic tissues (1). Compounds inactivated by glucuronic acid conjugation include steroid hormones, such as estrogens and catechol estrogens (2–5). Thus UGT enzymes may assist in the maintenance of steady-state levels of steroids in target tissues (6, 7). Moreover, there is evidence that one of the UGT1A family members, UGT1A1, is involved in E2 inactivation (8, 9) and expressed in the mammary gland (8), and, therefore, represents a good candidate gene in breast carcinogenesis.

Interindividual variation in UGT1A1 expression is explained by a polymorphic alteration in the atypical TATA-box region of the UGT1A1 gene (10–12). This polymorphic site is characterized by a variation in the number of TA repeats in the A(TA)n TAA sequence of the promoter. The most common allele (UGT1A1*) contains six TA repeats, whereas the principal variant allele (UGT1A1*28) contains seven TA repeats. Two other UGT1A1 alleles, the UGT1A1*33 [A(TA)n TAA] allele and the UGT1A1*34 [A(TA)n TAA] allele, have been found almost exclusively in the African-American population (13, 14). In a previous study, we investigated the association between breast cancer and A(TA)n TAA and A(TA)n TAA UGT1A1 alleles, which have been shown to have lower transcriptional activity in vitro (8). In the CBCS study, the alleles A(TA)n TAA and A(TA)n TAA were associated with a 2-fold increase in the risk of developing breast cancer in premenopausal African-American women.

In the present study, we evaluated, among primarily Caucasian women, the relationship between UGT1A1 alleles and breast cancer risk in a nested case-control study within the NHS cohort. Because decreased transcription of the UGT1A1 gene has been found to be associated with increased plasma levels of substrates of the UGT1A1 protein, e.g., bilirubin, we also evaluated the relationship between the UGT1A1*28 allele and circulating estrogen levels and the interaction between UGT1A1 and CYP17 polymorphisms.

1 The abbreviations used are: UGT, UDP-glucuronosyltransferase enzyme; NHS1, Nurses’ Health Study 1; OR, odds ratio; CI, confidence interval; CYP17, cytochrome p450c17α; CBCS, North Carolina Breast Cancer Study; E2, estradiol; E1, estrone; BMI, body mass index; RR, relative risk.
plasma steroid hormone levels to evaluate the differences in hormone levels between the genotypes; this was limited to postmenopausal women. The UGT1A1*1/UGT1A1*1 group was used as the reference category. To examine the interactive effect of the UGT1A1 gene and the CYP17 gene on hormone levels, we used the SAS Proc Mixed procedure. The natural logarithm of the plasma hormone values was used in the analyses to reduce the skewness of the regression residuals. We used the SAS statistical package for all analyses (SAS Institute, Inc.).

Results

Five controls and two cases could not be genotyped for UGT1A1 promoter polymorphisms. In addition, because of their very low prevalence, women who had at least one UGT1A1*28 allele [A(TA),TAA; n = 2; one case and one control and one UGT1A1*28 allele [A(TA),TA; n = 1; one case and one control] were not included in the analysis. Thus our study included 455 breast cancer cases and 609 controls. A total of 352 cases and 498 controls were postmenopausal, and 63 cases and 68 controls were premenopausal (Table 1); menopausal status was uncertain for 40 cases and 43 controls.

The prevalence of the homozygous UGT1A1*28 genotype in the present population was very close to that observed in a population of healthy Europeans [9% in NHS cases and 10% in NHS controls compared with 11.3% in Beutler et al. (13)]. The calculated frequencies for the UGT1A1*1 and UGT1A1*28 alleles were 0.68 and 0.32, respectively, in the controls and 0.69 and 0.31, respectively, in the cases. Genotype frequencies were in Hardy-Weinberg equilibrium for cases (χ2 = 1.49; degrees of freedom (df), 1; P = 0.22) and controls (χ2 = 0.80; df, 1; P = 0.37). The prevalence of the UGT1A1*23 and UGT1A1*34 alleles was extremely low in the NHS, which was consistent with the fact that most women self-reported their ethnicity as Caucasian, with very few Asians (0.3%), Hispanics (0.1%), and African Americans (0.3%). The prevalence of the lower-transcriptional activity alleles was slightly higher in the African-American population (0.40 in the CBCS control group; Ref. 8) compared with the Caucasian population (0.32 for the NHS control group).

There was no significant association between the low-activity allele, UGT1A1*28 [A(TA),TAA], and breast cancer (Table 1). Compared with women homozygous for the UGT1A1*1 allele, the estimated RR for the UGT1A1*28/28 genotype was 0.80 (CI, 0.49–1.29). A slightly but nonsignificantly higher RR was observed among premenopausal women (UGT1A1*28/28 versus *1/*1; RR = 1.28; CI, 0.38–4.64) compared with postmenopausal women (RR = 0.80; CI, 0.47–1.36). No association was revealed in subgroups defined by age at menarche, menopausal status, and clinical and pathological characteristics. We observed that having both polymorphisms in UGT1A1 (*28 and *28) and CYP17 (A2 polymorphism) did not affect the risk of developing breast cancer.

In the analysis of the relationship of genotype with plasma steroid hormone levels, we calculated least-square geometric mean levels for each UGT1A1 genotype among postmenopausal women not currently using postmenopausal hormones (Table 2). Compared with women with the UGT1A1*1/UGT1A1*1 genotype, women with the UGT1A1*1/UGT1A1*28 and UGT1A1*28/UGT1A1*28 subjects combined, because a gene dosage effect on breast cancer risk was not apparent. Unconditional multivariable models controlling for the matching factors enabled all controls to be included in analyses, limiting the cases to specified histopathological characteristics. Interactions between genotypes and breast cancer risk factors were evaluated by including interaction terms in multivariable logistic regression models. The likelihood ratio test was used to assess the statistical significance of these interactions.

Mixed regression models were used to evaluate the association between genotype and circulating hormone levels among controls, controlling for BMI at blood draw and the matching variables (16). Hormone fractions were measured in two to three different batches; the laboratory batch was treated as a random variable in all analyses except for dehydroepiandrosterone sulfate among never-users of hormone replacement, where a batch effect was not observed. We calculated least-square mean
analyses limited to women with a BMI limited to women with a BMI.

Table 1

<table>
<thead>
<tr>
<th>UGT1A1 genotype</th>
<th>Cases</th>
<th>Controls</th>
<th>RR</th>
<th>Adjusted RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>All women</td>
<td>n = 455 (%)</td>
<td>n = 609 (%)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>*1/*1</td>
<td>218 (48)</td>
<td>276 (45)</td>
<td>0.93 (0.71–1.21)</td>
<td>0.92 (0.69–1.22)</td>
</tr>
<tr>
<td>*1/*28</td>
<td>196 (43)</td>
<td>272 (45)</td>
<td>0.85 (0.54–1.33)</td>
<td>0.80 (0.49–1.29)</td>
</tr>
<tr>
<td>*28/*28</td>
<td>41 (9)</td>
<td>60 (10)</td>
<td>0.87 (0.60–1.23)</td>
<td>0.74 (0.46–1.19)</td>
</tr>
<tr>
<td>*1/*28 + *28/*28</td>
<td>237 (52)</td>
<td>332 (55)</td>
<td>0.91 (0.71–1.18)</td>
<td>0.90 (0.68–1.19)</td>
</tr>
</tbody>
</table>

Premenopausal women

- UGT1A1*1: 236 (45) vs. 304 (44) (0.85 (0.58–1.23))
- UGT1A1*28: 32 (8) vs. 47 (8) (0.91 (0.43–1.94))
- UGT1A1*28/*28: 17 (3) vs. 24 (4) (0.86 (0.46–1.61))

Postmenopausal women

- UGT1A1*1: 172 (49) vs. 222 (45) (1.0)
- UGT1A1*28: 26 (41) vs. 30 (44) (0.91 (0.77–1.13))
- UGT1A1*28/*28: 26 (51) vs. 30 (54) (0.84 (0.64–1.12))

Table 2

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Genotypesa</th>
<th>Overallb</th>
<th>BMI ≤23c</th>
<th>BMI &gt;23 ≤27c</th>
<th>BMI &gt;27d</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1-sulfate (pg/ml)</td>
<td>*1/*1</td>
<td>196.1 (111)</td>
<td>169.1 (35)</td>
<td>166.9 (34)</td>
<td>262.4 (42)</td>
</tr>
<tr>
<td>E1 (pg/ml)</td>
<td>*1/*1 or *28/*28</td>
<td>187.7 (146)</td>
<td>142.6 (38)</td>
<td>176.9 (57)</td>
<td>234.6 (51)</td>
</tr>
<tr>
<td>E2 (pg/ml)</td>
<td>*1/*1</td>
<td>29.3 (121)</td>
<td>26.0 (36)</td>
<td>27.3 (38)</td>
<td>34.1 (47)</td>
</tr>
<tr>
<td>E2</td>
<td>*1/*1 or *28/*28</td>
<td>30.6 (153)</td>
<td>24.6 (38)</td>
<td>28.0 (60)</td>
<td>37.2 (55)</td>
</tr>
</tbody>
</table>

E1-sulfate (pg/ml)

- *1/*1 and A1/A1 | 196.7 (42) | 191.3 (9) | 189.3 (16) | 230.6 (17) |
- *1/*1 or *28/*28 and A1/A2 or A2/A2 | 172.7 (96) | 121.5 (22) | 178.2 (48) | 206.4 (26) |
- E1 (pg/ml) | *1/*1 and A1/A1 | 28.5 (46) | 25.8 (9) | 29.6 (17) | 29.5 (20) |
- *1/*1 or *28/*28 and A1/A2 or A2/A2 | 30.2 (101) | 23.3 (22) | 27.8 (49) | 39.3 (30) |
- E2 (pg/ml) | *1/*1 and A1/A1 | 6.9 (45) | 5.4 (8) | 6.7 (17) | 8.6 (20) |
- *1/*1 or *28/*28 and A1/A2 or A2/A2 | 7.5 (101) | 5.6 (22) | 6.4 (49) | 11.0 (30) |

a Participants with missing hormone levels removed from analysis.
b Controlling for age, date of blood draw, BMI, and laboratory batch.
c Controlling for age, date of blood draw, time of blood draw, fasting status, BMI, and laboratory batch.

kg/m²). Comparing women with low (BMI ≤23 kg/m²) versus high (BMI ≥27 kg/m²) estrogen production, the elevation of circulating E₂ levels was greater in women who had at least one UGT1A1*28 allele (87.9%) compared with UGT1A1*1 homozygous women (52.4%). Within BMI subgroups, women who had at least one UGT1A1*28 allele in the highest stratum of BMI (≥27 kg/m²) had a nonsignificant increase in the level of E₂ (17.5%; P = 0.09) compared with UGT1A1*1 homozygous women (Table 2). Furthermore, we evaluated the combination of UGT1A1 and CYP17 genotypes on hormone levels. In analyses limited to women with a BMI >27 kg/m² who had at least one UGT1A1*28 and one CYP17 A2 allele (n = 30), there was a marginally significant increase in the levels of E₂ (33.2%; P = 0.07) and a nonsignificant elevation in E₁ (27.9%; P = 0.18) compared with UGT1A1*1 and CYP17 A1 homozygous carriers (n = 20).

Discussion

An important role for the UGT enzymes is to maintain intracellular steady-state levels of steroids, including estrogens, in target tissues (6, 7). Accordingly, high levels of estrogens...
glucuronides have been observed in breast cyst fluid, suggesting their formation within the mammary gland (17, 18). We hypothesized that alteration in the glucuronidation pathway, which directly inactivates estrogens and catechol metabolites at their site of action (2–5), may potentially modify estrogen concentration and consequently estrogen-related cancer risk. Functional analyses of the transcriptional promoter activity demonstrated that allele UGT1A1*28 has a 30% decrease in transcription and that the UGT1A1*34 allele has a 50% decreased transcription, whereas the allele UGT1A1*33 has a 20% increased transcription compared with the UGT1A1*1 wild-type allele (8). Additional studies based on phenotypic measurements revealed that those who were carriers of at least one UGT1A1*28 allele had much lower glucuronidation rates compared with homozygous UGT1A1*1 (19, 20). On the basis of these functional studies (8, 13), women who had at least one low-transcription allele [ULTG1A1*28 [A(TA)6-TAA], alone or compared with women who had high-activity alleles UGT1A1*1 [A(TA)6-TAA]. The present study extends previous work among African-American women (200 cases and 200 controls) in the CBCS. In the CBCS study, the low-activity UGT1A1 alleles were positively associated with invasive breast cancer among premenopausal women (OR, 1.8: 95% CI, 1.0–3.1; P = 0.06; Ref. 8), particularly among women with estrogen receptor-negative breast cancer (OR, 2.1: 95% CI, 1.0–4.2; P = 0.04) compared with estrogen receptor-positive breast cancer (OR, 1.3: 95% CI, 0.6–3.0; P = 0.5). In contrast, in the present NHS, a larger population-based prospective study, no influence of the UGT1A1 polymorphism was observed on the susceptibility to breast cancer. Furthermore, we found no interaction between the UGT1A1*28 and the CYP17 A2 allele in relation to breast cancer risk.

In the analysis of hormone levels and UGT genotype among postmenopausal women not taking hormone replacement therapy, we observed a moderate increase in the levels of E1 and E2, associated with the UGT1A1*28 allele alone and in combination with CYP17 A2 polymorphism only in overweight women (BMI > 27 kg/m2). However, this modest elevation in the plasma levels of estrogenic hormones in postmenopausal women may be insufficient for us to detect an altered risk of breast cancer. It is also well known that different UGT enzymes possess overlapping substrate specificity, including reactivity for estrogenic hormones (5). In the case of E2 glucuronidation, although UGT1A1 is the major UGT reported to date to actively conjugate E2 (8, 9), other existing or not yet isolated UGTs may have a higher specificity and/or efficacy toward this steroid molecule (2, 21). In that case, a partial deficiency in UGT1A1, such as the one studied here, would have a limited impact on E2 metabolism and, consequently, on breast cancer risk. The modest elevations of E2 observed in the heaviest women who are carriers of at least one UGT1A1*28 allele is suggestive of a possible contribution of the glucuronidation pathway, and especially of UGT1A1, in the maintenance of hormone homeostasis in situations where estrogens are present at higher concentrations.

Our results suggest that the decrease in transcription of the UGT1A1 gene caused by the TA polymorphism is not sufficient to alter breast cancer risk in Caucasian women.

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References

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