Functional Significance of UDP-Glucuronosyltransferase Variants in the Metabolism of Active Tamoxifen Metabolites

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Abstract
Tamoxifen (TAM) is a selective estrogen receptor modulator widely used in the prevention and treatment of breast cancer. A major mode of metabolism of the major active metabolites of TAM, 4-OH-TAM and endoxifen, is by glucuronidation via the UDP-glucuronosyltransferase (UGT) family of enzymes. To examine whether polymorphisms in the UGT enzymes responsible for the glucuronidation of active TAM metabolites play an important role in interindividual differences in TAM metabolism, cell lines overexpressing wild-type or variant UGTs were examined for their activities against TAM metabolites in vitro. For variants of active extrahepatic UGTs, the UGT1A1(134A/277Tyr) variant exhibited no detectable glucuronidation activity against the trans isomers of either 4-OH-TAM or endoxifen. Little or no difference in TAM glucuronidating activity was observed for the UGT1A1(134Gly/277Tyr) variants compared with their wild-type counterparts. For active hepatic UGTs, the UGT2B7(268Tyr) variant exhibited significant (P < 0.01) 2- and 5-fold decreases in activity against the trans isomers of 4-OH-TAM and endoxifen, respectively, compared with wild-type UGT2B7(268His). In studies of 111 human liver microsomal specimens, the rate of O-glucuronidation against trans-4-OH-TAM and trans-endoxifen was 28% (P < 0.001) and 27% (P = 0.002) lower, respectively, in individuals homozygous for the UGT2B7 Tyr268Tyr genotype compared with subjects with the UGT2B7 His268His genotype, with a significant (P < 0.01) trend of decreasing activity against both substrates with increasing numbers of the UGT2B7(268Tyr) allele. These results suggest that functional polymorphisms in TAM-metabolizing UGTs, including UGT2B7 and potentially UGT1A8, may be important in interindividual variability in TAM metabolism and response to TAM therapy.

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Introduction
1-[4-(2-Dimethylaminoethoxy)-phenyl]-1,2-diphenylbut-1-(Z)-ene [tamoxifen (TAM)] is a nonsteroidal antiestrogen that has been commonly used for the treatment and prevention of estrogen-dependent breast cancer (1–4). Adjuvant TAM treatment increases recurrence-free survival and overall survival in breast cancer patients with hormone receptor–positive tumors irrespective of their nodal status, menopausal status, or age (5). In addition to its antiestrogenic properties, which have been related to symptoms such as hot flashes, vaginal bleeding, and pruritus vulvae (2, 6), TAM also has partial estrogen-agonistic effects that may be linked to reduced risk for ischemic heart disease and osteoporosis (7, 8), but may also increase the risk for endometrial cancer (9, 10) and venous thromboembolism (11). Although TAM is generally well tolerated, there is significant interindividual variability in the clinical efficacy as well as toxicities of TAM. For instance, ~30% of patients acquire TAM resistance and relapse (12). In addition, the relative risk of endometrial cancer in patients treated with TAM is estimated to be 2- to 3-fold that of controls (10, 13–15). The mechanisms underlying variability in response to TAM and to TAM-related toxicities remains obscure. Because there is compelling evidence that TAM is converted to antiestrogenic metabolites that are more potent than TAM itself, altered patterns of metabolism of TAM and/or its active metabolites might contribute to this interindividual variability.

TAM is metabolized via cytochrome P450s, primarily CYP2D6 and CYPs 3A4/3A5, into several metabolites after oral administration, including the hydroxylated TAM metabolites 4-OH-TAM and endoxifen. Both 4-OH-TAM and endoxifen are abundant in the serum of TAM-treated patients, with the levels of serum endoxifen approaching 6- to 12-fold that observed for 4-OH-TAM. Because the trans isomers of both 4-OH-TAM and endoxifen exhibit up to 100-fold the levels of antiestrogenic activity compared with TAM (16–21), it is thought that they may be the major contributors to the antiestrogenic properties of TAM. Whereas cis-4-OH-TAM exhibits antiestrogenic activity in vitro in the presence of estradiol, it has also been suggested to possess some estrogen agonist activity (22–24). The trans isomers of 4-OH-TAM and endoxifen are more abundant than the cis isomers, possibly at a ratio of 70:30, at physiologic pH (25, 26).

An important route of elimination and detoxification of TAM and its metabolites is via glucuronidation. TAM is excreted predominantly through the bile primarily by conjugation to glucuronic acid (27), with most of the 4-OH-TAM found in the bile of TAM-treated patients as a glucuronide conjugate (27, 28). TAM glucuronides have also been identified in the urine and serum of TAM-treated patients (27, 28), and it has been suggested that glucuronidation within target tissues like the adipose tissue of the breast may also be important in terms of TAM metabolism and overall TAM activity (29).

N-glucuronidation occurs for both TAM and 4-OH-TAM, whereas O-glucuronidation occurs for 4-OH-TAM and endoxifen (30, 31). In vitro studies have shown that the hepatic UGT1A4 is the only active enzyme responsible for the N-glucuronidation of TAM and 4-OH-TAM, whereas UGT2B7 and, to a lesser extent, UGT1A1 are the major hepatic enzymes involved in the O-glucuronidation of the trans isomers of 4-OH-TAM and endoxifen (30, 31). UGT2B7 exhibited higher levels of activity against the trans isomers of...
4-OH-TAM and endoxifen; other hepatic UGTs (including UGTs 1A3, 1A9, 2B15, and 2B17) were significantly more active against cis TAM metabolites (31). The extrahepatic UGTs 1A10 and 1A8 are expressed in target tissues, including breast, and were also shown to be highly active against isomers of 4-OH-TAM and endoxifen in vitro (31). Whereas previous studies have shown that the UGT1A4*48 Val variant exhibits increased N-glucuronidation activity in vitro against 4-OH-TAM compared with the wild-type UGT1A4*48 Val isoform (30), no studies have been performed examining UGT variants and O-glucuronidation activity against 4-OH-TAM or endoxifen. The goal of the present study was to examine whether prevalent missense single nucleotide polymorphisms (SNP) in the most active TAM metabolite O-glucuronidating enzymes alters their activity against the trans isomers of 4-OH-TAM and endoxifen and could therefore potentially play an important role in patient response to TAM.

Materials and Methods

Chemicals and materials. Trans-4-OH-TAM (98% pure), UDPGA, alamethicin, β-glucuronidase, and bovine serum albumin were purchased from Sigma-Aldrich. Endoxifen was synthesized in the Organic Synthesis Facility at the Penn State College of Medicine, with the trans-endoxifen isomer purified as described previously (31). High-performance liquid chromatography (HPLC) grade ammonium acetate, acetonitrile, and peptide synthesis grade triethylamine were purchased from Fisher Scientific and were used after filtration. DMEM, Dulbecco’s PBS (minus magnesium chloride and calcium chloride), fetal bovine serum, penicillin-streptomycin, and geneticin (G418) were purchased from Life Technologies. The Platinum Pfx DNA polymerase and the pCDNA3.1/V5-His-TOPO mammalian expression vector were obtained from Invitrogen, whereas restriction enzymes DpnI and Stul were purchased from New England Biolabs. The BCA protein assay kit was purchased from Pierce, whereas the QIAEX II gel extraction kit was purchased from Qiagen. The human UGT1A Western blotting kit and anti-UGT1A antibody were purchased from Gentest. All other chemicals used were purchased from Fisher Scientific unless otherwise specified.

UGT-overexpressing cell lines. The HEK293 cell lines overexpressing the wild-type UGT1A10139Glu, UGT2B7268His, and UGT1A8173Ala/277Cys isoforms and the UGT1A10139Glu and UGT2B7268His variants used in this study have been described previously (32–34). The UGT1A10139Glu and UGT2B7268His variants were generated by site-directed mutagenesis of the pCDNA3.1/V5-His-TOPO plasmid expressing the wild-type UGT1A gene as previously described (31, 33) using the QuikChange Site-Directed Mutagenesis Kit (Stratagene). The primers used to change UGT1A codon 173 from Ala to Gly were as follows: sense, 5′-TTTACTAATTTTTGGC-CATTGCAGGAG-3′, and antisense, 5′-CTCTCGATCAATGCGAATACAAA-TAAGGTATCA-3′, corresponding to nucleotides 349 to 377 relative to the translation start site. The primers used to change UGT2B7 codon 277 from Cys to Tyr were sense, 5′-GGGTTCTACATCATGACGAAAAAGC-3′, and antisense, 5′-GTGTTCTCCTGATGTTGATGACC-3′, corresponding to nucleotides +815 to +843 relative to the translation start site. The underlined base for each primer indicates the base pair change. Similar to that described previously for site-directed mutagenesis–generated UGT variants (31, 33), the UGT1A10139Glu and UGT2B7268His cDNA sequences were confirmed by dideoxy sequencing before transfection by electroconversion into the HEK293 (human embryonic kidney fibroblast) cell line as previously described (31, 33). Cells were grown in DMEM to 80% confluence before the preparation of cell homogenates as previously described (34). Total homogenate protein concentrations were measured using the BCA protein assay.

UGT protein levels were determined by Western blot analysis for all UGT-overexpressing cell lines examined in this study as previously described (33). For UGT1A-overexpressing cells, the UGT1A antibody from Gentest was used; for UGT2B-overexpressing cells, a previously described UGT2B-specific antibody was used (31). Relative UGT protein levels were expressed as the mean of three independent experiments, and all activity assays were normalized relative to UGT expression in the respective UGT-overexpressing cell line.

Human liver microsomes. Normal human liver tissue specimens (n = 111) were obtained from the Tissue Procurement Facility at the H. Lee Moffitt Cancer Center (Tampa, FL) and include 78 liver specimens that were prepared at 4°C in 50 mmol/L Tris-HCl (pH 7.4), 10 mmol/L MgCl2, 4 mmol/L UDPGA, and 1 to 250 μmol/L trans-4-OH-TAM or 8 to 725 μmol/L endoxifen for cell homogenate glucuronidation assays. Kinetic analysis of HLM from subjects with varying UGT2B7 genotypes was performed using 0.5 to 15 μmol/L trans-4-OH-TAM and 2 to 60 μmol/L endoxifen in five HLM specimens from individual subjects from each UGT2B7 genotype group (15 HLM specimens total). Reactions were terminated by the addition of 100 μL cold methanol on ice. Mixtures were centrifuged for 10 min at 4°C at 16,100 × g before the collection of the supernatant. All glucuronidation assays were performed in triplicate for cell homogenate assays and in duplicate for HLM assays in independent experiments.

TAM metabolites were assessed for cell homogenous glucuronidation assays by HPLC identical to that described previously (30, 31). Assays of TAM metabolite formation in HLM were analyzed by ultra performance liquid chromatography–tandem mass spectrometry (UPLC/MS/MS) using a Waters Acquity UPLC consisting of a binary gradient pump, an autosampler (maintained at 4°C), and a UV detector operated at 254 nm, attached to a Waters TQD triple quadrupole mass spectrometer that was purchased after the analysis of UGT-overexpressing cell lines (described above). Similar to that described previously (26), each sample was injected onto an Acquity UPLC BEH C18 1.7 μmol/L, 2.1 × 100 mm column (Waters) with the following gradient elution conditions for trans-4-OH-TAM: starting with 69% buffer A (0.01 mol/L NH4Ac (pH 5.0) /31% acetonitrile for 2 min with a subsequent linear gradient to 75% acetonitrile over 2 min. The gradient elution conditions for trans-endoxifen, using the same buffers, were as follows: starting with 30% acetonitrile for 4 min and a subsequent linear gradient to 75% acetonitrile for 2 min. The elution flow rate was 0.5 mL/min and 5 μL of the reaction was injected for all assays. Electrospray ionization mass spectrometry daughter scans of 564 and 550 (m/z) verified the glucuronides of trans-4-OH-TAM and trans-endoxifen. The formation of 4-OH-TAM and endoxifen glucuronides were quantified by UPLC based on the ratio of the glucuronide versus free trans-4-OH-TAM or trans-endoxifen. HLM assays without TAM metabolite were regularly analyzed as negative controls for glucuronidation activity as previously described (34).

For 4-MU, glucuronidation assays were performed as described above with an incubation time of 120 min. HPLC analysis used the following gradient program: starting with 98% buffer A [100 mmol/L NH4Ac (pH 5.0) /31% acetonitrile for 2 min with a subsequent linear gradient to 75% acetonitrile over 17.5 min] and then maintained at 70% for 10 min.

UGT genotyping. Genomic DNA from the 111 liver specimens examined for glucuronidation activity in this study were used to genotype the UGT2B7 codon 268 (His>Tyr) polymorphism, the UGT1A1*28 TATA box polymorphism (see Genbank wild-type accession number NM_000463.
and SNP rs8175347), and the UGT1A4 codon 24 (Pro-Thr) and codon 48 (Leu-Val) polymorphisms. UGT1A4 genotypes were determined by direct sequencing of PCR-amplified PCR products spanning both codons 24 and 48 for UGT1A4. The same primers were used for both PCR amplification and sequencing of UGT1A4: sense, 5'-GGCTTCTGCTGATGGCCGAC-3', and antisense, 5'-CCTTGAGTGTAGCCCAGCGT-3', corresponding to nucleotides located at +13 to +8 and +277 to +306, respectively, relative to the UGT1A4 translation start site (Genbank accession no. NM_007120). Sequencing was performed using an ABI 3130 Capillary Sequencer at the Functional Genomics Core Facility at the Penn State College of Medicine.

The UGT1A1*28 polymorphism was genotyped using DNA fragment analysis by capillary electrophoresis on the ABI 3130 Capillary Sequencer at the Penn State Molecular Genetics Core Facility using primers and PCR conditions similar to those described previously (36) using 0.5 μL of a size standard (GeneScan 500 LIZ Size Standard, Applied Biosystems) as a DNA size marker. Informative results were obtained for 105 of the 111 liver specimens examined in this study.

UGT2B7 codon 268 genotypes were determined primarily by real-time PCR assays using the TaqMan Drug Metabolism Genotyping Assay C_32449942_20 (Applied Biosystems) in the ABI 7900HT sequence detection system equipped with an autoloader in the Functional Genomics Core Facility at the Penn State College of Medicine. Forty percent of all samples within each of the three potential UGT2B7 genotype groups (His268His, His268Tyr and Tyr268Tyr; as identified by real-time PCR) were further confirmed by standard PCR and direct sequencing. The same primers were used for both PCR and sequencing. sense, 5'-CTATATCCTTTACACTTCTATAGTGCTTTACTTT-3' and antisense, 5'-GCTAGAAAAGCAAAGAAGGGAAAAAATGAT-TATTTTTCCCTGTA-3'.

Sequencing was performed using an ABI 3130 Capillary Sequencer at the Functional Genomics Core Facility at the Penn State College of Medicine using primers and PCR conditions similar to those described previously (36) using 0.5 μL of a size standard (GeneScan 500 LIZ Size Standard, Applied Biosystems) as a DNA size marker. Informative results were obtained for 105 of the 111 liver specimens examined in this study.

UGT2B7 expression analysis. Matching total RNA was available for expression analysis for 99 of the 111 liver specimens analyzed in this study. Five micrograms of RNA were used for cDNA synthesis using standard reverse transcription methods as described above, with 20 ng of cDNA used for expression analysis using the ABI gene expression kit assay for UGT2B7 (HS02556232_s1; Applied Biosystems) with the ABI 7900HT detection system equipped with an autoloader in the Functional Genomics Core Facility at the Penn State College of Medicine. Expression assays were performed in triplicate with expression normalized relative to the housekeeping gene -Actin.

Results

Kinetik studies of TAM metabolite glucuronidation by UGT variants. Results from previous studies showed that the hepatic UGT2B7 and the extrahepatic UGTs 1A8 and 1A10 exhibited the highest overall O-glucuronidating activities against trans-4-OH-TAM and trans-endoxifen (31). Known missense polymorphisms have been identified for each of these UGTs, including a highly prevalent SNP at codon 268 of the UGT2B7 gene (32), a codon 139 SNP in the UGT1A10 gene that is most prevalent in African Americans (37), and two coding region SNPs that result in amino acid changes of Ala to Gly at codon 173 and Cys to Tyr at codon 277 of the UGT1A8 gene (38). To determine whether any of these SNPs result in differential activities against the trans isomers of 4-OH-TAM and endoxifen, in vitro kinetic analysis of HEK293 cells overexpressing the wild-type or variant isoforms of each of these three UGT enzymes was performed. Whereas the levels of expression of wild-type versus variant UGT1A10 protein was determined for UGT1A10-overexpressing cell lines in previous studies (33), semiquantitative Western blot analysis was performed for the UGT1A8- and UGT2B7-overexpressing cell lines. As shown in Fig. 1, each of the UGT1A8-overexpressing cell lines developed for the present analysis exhibited similar levels of expression (8.0, 6.3, and 7.0 ng UGT1A8/μg total protein for the UGT1A8*10/Ax/277Cys-, UGT1A8*173Ala/277Tyr-, and UGT1A8*173Ala/277Tyr-overexpressing lines, respectively). As described previously in other laboratories for the UGT2B7-overexpressing cell lines (32), UGT2B7*268His was consistently expressed at a level that was 3.3-fold that of the UGT2B7*268Tyr variant in the present analysis (results not shown). These values were used for normalization of UGT1A8 and UGT2B7 levels in their respective UGT-overexpressing cell lines in in vitro kinetic analysis.

Representative HPLC traces of glucuronidation formation by UGT-overexpressing cell homogenates are shown in Fig. 2. As described previously for cell lines overexpressing wild-type UGTs 1A8, 1A10, or 2B7 (31), significant levels of O-glucuronidation of the trans isomers of 4-OH-TAM and endoxifen were observed in the present study; no N-glucuronidation of these TAM metabolites was observed for these UGTs. The UGT1A8*173Ala/277Cys variant exhibited no difference in overall glucuronidation activity (Vmax/Km) against trans-4-OH-TAM and exhibited a small (1.25-fold)
but significant ($P < 0.05$) decrease in overall activity (manifested primarily by a higher $K_m$) against trans-endoxifen compared with wild-type UGT1A8173Ala/277Cys (Table 1). In contrast, the UGT1A8173Ala/277Tyr variant exhibited no detectable glucuronidation against the trans isomers of either 4-OH-TAM or endoxifen (Table 1). However, the UGT1A8173Ala/277Tyr variant exhibited detectable levels of activity against 4-MU (Fig. 2D). No difference in overall glucuronidation activity was observed for the UGT1A10139Lys variant versus wild-type UGT1A10 against the trans isomers of 4-OH-TAM and endoxifen.

Kinetic analysis showed that significantly higher glucuronidation activities were observed for the wild-type UGT2B7268His compared with the UGT2B7268Tyr variant against the trans isomers of both 4-OH-TAM ($P < 0.05$) and endoxifen ($P < 0.01$; Table 1). This was manifested by a higher $K_m$ (2.4-fold) and a lower $V_{max}/K_m$ (2.4-fold) for 4-OH-TAM, as well as a lower $V_{max}$ (5.5-fold) and lower $V_{max}/K_m$ (5.0-fold) for endoxifen.

Glucuronidation activities of HLM against the trans isomers of 4-OH-TAM and endoxifen stratified by UGT genotypes. To determine the rate of glucuronidation of trans-4-OH-TAM and endoxifen.

**Figure 2.** HPLC analyses of overexpressing cell line glucuronidating activities. HPLC traces of trans-TAM-4-O-glucuronide and trans-endoxifen-O-glucuronide formation by (A) UGT1A8173Ala/277Cys-, (B) UGT1A10139Glu-, and (C) UGT2B7268His-overexpressing HEK293 cells using 500 μg homogenate protein in glucuronidation activity assays as described in Materials and Methods. D, 4-MU-O-glucuronide formation using 1 mg UGT1A8173Ala/277Tyr-overexpressing cell homogenate. Overexpressing cell homogenates were incubated at 37°C for 60 or 120 min for assays with TAM metabolites or 4-MU, respectively, before analysis by HPLC as described in Materials and Methods. Peak 1, trans-4-TAM-O-glucuronide; peak 2, trans-4-OH-TAM; peak 3, trans-endoxifen-O-glucuronide; peak 4, trans-endoxifen; peak 5, 4-MU-O-glucuronide; peak 6, 4-MU.
trans-endoxifen, glucuronidation assays were performed for 111 HLMs and analyzed by UPLC/MS/MS. The concentrations of 4-OH-TAM or endoxifen used in the HLM glucuronidation activity assays was determined after kinetic analysis of three randomly chosen HLM specimens—the resulting \( K_m \) values were 4 and 30 \( \mu \text{mol/L} \) for trans-4-OH-TAM and trans-endoxifen, respectively (data not shown). Using 4 \( \mu \text{mol/L} \) trans-4-OH-TAM as substrate in HLM glucuronidation activity assays, two major putative glucuronide peaks were observed, the TAM-4-O-glucuronide and the 4-OH-TAM-N"-glucuronide, which exhibited retention times of 1.76 and 3.35 min, respectively, distinct from free trans-4-OH-TAM, which eluted at 3.95 min (Fig. 3A). Using 30 \( \mu \text{mol/L} \) trans-endoxifen as substrate in HLM glucuronidation activity assays, a single major putative glucuronide peak was observed at a retention time of 1.95 min, which was distinct from the endoxifen peak eluting at 3.90 min (Fig. 3B). All putative glucuronide peaks were sensitive to treatment with β-glucuronidase (results not shown), eluted at retention times identical to previously characterized TAM-glucuronide standards (ref. 26; results not shown), and were confirmed by tandem MS (Fig. 3C and D; the MS/MS pattern observed for trans-TAM-4-O-glucuronide and trans-4-OH-TAM-N"-glucuronide are identical). For 4-OH-TAM glucuronidation assays, a third, smaller peak eluting at a retention time of 2.02 min was confirmed to be cis-TAM-4-O-glucuronide, likely formed due to spontaneous interconversion between the trans and cis 4-OH-TAM isomers (34). Similar to that observed in previous studies for three HLM specimens (31), no endoxifen-N-glucuronide was observed with any of the 111 HLM examined in the present analysis. The mean rates of formation of TAM-4-O-glucuronide, 4-OH-TAM-N"-glucuronide, and endoxifen-4-O-glucuronide in HLM were 141 ± 45, 175 ± 52, and 168 ± 66 \( \mu \text{mol-min}^{-1} \cdot \text{mg}^{-1} \), respectively. A 4.5-, 10-, and 17-fold range in glucuronide formation was observed for TAM-4-O-glucuronide, 4-OH-TAM-N"-glucuronide, and endoxifen-4-O-glucuronide, respectively. The range of the ratio of TAM-4-O-glucuronide to 4-OH-TAM-N"-glucuronide in the HLM samples was 8.0-fold.

As described above, previous studies have indicated that UGT2B7 is the major hepatic enzyme that performs O-glucuronidation of the trans isomers of both 4-OH-TAM and endoxifen (31). When stratifying HLM O-glucuronidation activities by UGT2B7 codon 268 genotype, there was a near-significant (\( P = 0.059 \)) 13% decrease in TAM-4-O-glucuronide formation in HLM with the UGT2B7 (His268Tyr) genotype and a significant (\( P < 0.001 \)) 28% decrease in TAM-4-O-glucuronide formation in HLM with the UGT2B7 (Tyr268Tyr) genotype compared with HLM with the UGT2B7 (His268His) genotype (Fig. 4A). A significant (\( P = 0.01 \)) 17% decrease in TAM-4-O-glucuronide formation was observed in HLM with the UGT2B7 His268Tyr genotype versus HLM with the UGT2B7 (Tyr268Tyr) genotype. A significant trend of decreasing O-glucuronidation of trans-4-OH-TAM was observed in HLM with increasing numbers of the UGT2B7*268Tyr allele (\( P < 0.001 \)).

Table 1

| UGT variant | Trans-4-OH-TAM | | | Trans-endoxifen | |
|-------------|----------------|-----------------|-----------------|-----------------|
| V\(_{\text{max}}\) (\( \mu \text{mol/min/\mug} \))* | \( K_m \) (\( \mu \text{mol/L} \)) | \( V_{\text{max}}/K_m \) (\( \mu \text{L/min/\mug} \))* | V\(_{\text{max}}\) (\( \mu \text{mol/min/\mug} \))* | \( K_m \) (\( \mu \text{mol/L} \)) | \( V_{\text{max}}/K_m \) (\( \mu \text{L/min/\mug} \))* |
| UGT2B7*268His | 0.55 ± 0.18 | 3.7 ± 0.6 | 0.15 ± 0.03 | 3.0 ± 0.44 | 101 ± 17 | 0.030 ± 0.004 |
| UGT2B7*268Tyr | 0.54 ± 0.09 | 8.7 ± 0.8 | 0.062 ± 0.01 | 0.55 ± 0.01 | 101 ± 15 | 0.006 ± 0.001 |
| UGT1A1*139Gln | 4.7 ± 0.3 | 96 ± 8 | 0.049 ± 0.006 | 5.7 ± 0.7 | 40 ± 3 | 0.14 ± 0.005 |
| UGT1A1*139Yxe | 2.1 ± 0.2 | 52 ± 6 | 0.040 ± 0.006 | 1.9 ± 0.2 | 13 ± 2 | 0.14 ± 0.004 |
| UGT1A8*173A/277Cys | 2.3 ± 0.1 | 23 ± 2 | 0.10 ± 0.02 | 5.4 ± 0.2 | 98 ± 9 | 0.060 ± 0.004 |
| UGT1A8*173G/277Hy | 5.4 ± 0.2 | 43 ± 7 | 0.13 ± 0.03 | 5.9 ± 0.4 | 135 ± 26 | 0.040 ± 0.005 |

NOTE: All data are the mean ± SD based on three independent experiments. Homogenates from cells overexpressing UGT1A1*139Yxe exhibited no detectable activity against trans-4-OH-TAM and trans-endoxifen.

* Data are expressed per microgram of UGT protein as determined by Western blot analysis.

† \( P < 0.05 \), significant difference compared to wild-type isoform.

‡ \( P < 0.01 \), significant difference compared to wild-type isoform.
(*1/*28) genotypes (results not shown). No significant associations were observed for either the UGT1A4 codons 24 (Pro>Thr) or 48 (Leu>Val) polymorphisms and HLM N-glucuronidation activity against 4-OH-TAM (results not shown).

**Discussion**

The present study examines the potential role of UGT polymorphisms on the metabolism of the trans isomers of 4-OH-TAM and endoxifen, the major active metabolites of tamoxifen. Several UGTs were previously shown to be active against these metabolites, with UGT2B7 being the most active hepatic UGT. As UGT2B7 expression has been detected in a variety of tissues, including liver, the gastrointestinal tract, and breast (39–43), variations in UGT2B7 function or expression could potentially significantly affect individual response to drugs or chemotherapeutic agents. The data presented in this study show that O-glucuronidation of both trans-4-OH-TAM and trans-endoxifen in HLM was significantly

![Figure 3](image-url)
The UGT2B7 codon 268 genotypes, which were previously shown to be active against TAM, trans-4-OH-TAM, and/or trans-endoxifen (30, 31), were examined for their effect on HLM glucuronidation activity. A microsatellite (TA) repeat polymorphism present in the TATAAA box of the UGT1A1 promoter has been linked to lower UGT1A1 expression (45, 46) and lower activity against a variety of endogenous and exogenous substrates, including bilirubin (45, 47, 48), carcinogens such as metabolites of benzo(a)pyrene (45), and chemotherapeutic agents such as SN-38, the major metabolite of irinotecan (49, 50). The nonsignificant trend of decreased glucuronidation activity against TAM metabolites that was observed in HLMs from subjects with one or more UGT1A1*28 alleles is consistent with previous studies indicating that UGT1A1 exhibits only weak relative activity against these substrates compared with UGT2B7 (31) and may therefore play a more minor role in TAM metabolism.

The N-glucuronidation of TAM and 4-OH-TAM was previously shown to be performed exclusively by UGT1A4 (30, 51). Although previous studies showed that the UGT1A4 codon 48 polymorphism was linked to a small but significant alteration in N-glucuronidation activity against TAM and 4-OH-TAM in vitro (30), no significant difference was observed in HLM against trans-4-OH-TAM in the present study. This may be due to the relatively low number of HLM specimens that were heterozygous (n = 13) or homozygous (n = 1) for the UGT1A4*48Val variant in this study.

The extrahepatic UGTs 1A10 and 1A8 exhibited the highest levels of activity in vitro against the trans isomers of 4-OH-TAM and endoxifen in previous studies (31). In the present study, no effect on TAM metabolite glucuronidation activity was observed in vitro for the UGT1A1*139Val variant compared with wild-type UGT1A1*139Glu. Similarly, the UGT1A8*773Val variant exhibited a marginally significant lower overall in vitro glucuronidating activity against trans-endoxifen as determined by V_{max}/K_m compared with wild-type UGT1A8*773Glu; no significant difference was observed for this variant against trans-4-OH-TAM. This relatively minor effect on TAM metabolite glucuronidating activities is consistent with the fact that the Ala>Val amino acid substitution at UGT1A8 codon 173 is a conservative nonpolar amino acid change and with data from previous in vitro metabolic studies that revealed that UGT1A8*773Ala and UGT1A8*773Val exhibit similar catalytic properties (38, 52). Interestingly, the UGT1A8*277Val variant exhibited no detectable glucuronidating activity against both trans-4-OH-TAM and trans-endoxifen. This is consistent with previous data indicating that this variant exhibited dramatically reduced activity toward other substrates (38, 52). Although the prevalence of this polymorphism is low in the population (~2% in Caucasians; ref. 38), the observation that UGT1A8 is highly active against TAM metabolites and is well expressed in the breast (53, 54) suggests that, like the UGT2B7 codon 268 polymorphism, the UGT1A8 codon 277 polymorphism could potentially be important in individual response to TAM.

The results described above are consistent with that observed previously for functional polymorphisms in the CYP2D6 gene. Decreased levels of endoxifen were observed in the serum of individuals carrying the UGT2B7 expression or a change in activity against TAM metabolites compared with HLM containing the wild-type IVS1 + 985A (results not shown). These data suggest that the decrease in O-glucuronidation activity against TAM metabolites in HLM associated with the UGT2B7 codon 268 polymorphism is indeed due to functional changes within the UGT2B7 enzyme.
TAM-treated women at stratifying patients by the CYP2D6 deletion genotype (55), a decrease also observed when CYP2D6 inhibitors were coadministered with TAM (19). The CYP2D6*4 deletion allele has been associated with time until breast cancer recurrence, relapse-free survival, disease-free survival, and overall survival in patients treated with TAM (19, 55, 56). In addition, patients with the CYP2D6*4 genotype report few, if any, occurrences of hot flashes (55). These data suggest an important role for endoxifen in TAM therapeutic efficacy. Despite a strong correlation between the CYP2D6 genotype and serum levels of endoxifen in patients treated with TAM, large variability in circulating endoxifen levels are still observed after controlling for the CYP2D6 genotype (19, 55). The data described in the present study suggests that interindividual differences in TAM glucuronidation pathways may help explain this variability.

In summary, results from this study suggest that genetic variants in UGTs that are highly active against TAM metabolites significantly alter TAM metabolism in vitro and, potentially, its elimination in TAM-treated individuals. Similar to that described above for CYP2D6*4, this could potentially affect overall patient response to TAM. Additional studies examining the effect of UGT1A8 and UGT2B7 genotypes on breast microsomal glucuronidation activity against TAM metabolites, plasma TAM metabolite levels, and overall patient response to TAM will be required to further examine the role of UGT polymorphisms on the therapeutic efficacy of TAM.

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Functional Significance of UDP-Glucuronosyltransferase Variants in the Metabolism of Active Tamoxifen Metabolites

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