Potential Role of *UGT1A4* Promoter SNPs in Anastrozole Pharmacogenomics

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ABSTRACT

Anastrozole belongs to the nonsteroidal triazole-derivative group of aromatase inhibitors. Recently, clinical trials demonstrated improved antitumoral efficacy and a favorable toxicity with third-generation aromatase inhibitors, compared with tamoxifen. Anastrozole is predominantly metabolized by phase I oxidation with the potential for further phase II glucuronidation. It also, however, is subject to direct *N*-glucuronidation by UDP-glucuronosyltransferase 1A4 (*UGT1A4*). Anastrozole pharmacokinetics vary widely among patients, but pharmacogenomic studies of patients treated with anastrozole are sparse. In this study, we examined individual variability in the glucuronidation of anastrozole and its association with *UGT1A4* promoter and coding region polymorphisms. In vitro assays using liver microsomal preparations from individual subjects (*n* = 96)

demonstrated 235-fold variability in anastrozole glucuronidation. Anastrozole glucuronidation was correlated ($r = 0.99$; $P < 0.0001$) with lamotrigine glucuronidation (a diagnostic substrate for *UGT1A4*) and with *UGT1A4* mRNA expression levels in human liver microsomes ($r = 0.99$; $P < 0.0001$). Recombinant *UGT1A4* catalyzed anastrozole glucuronidation, which was inhibited by hecogenin ($IC_{50} = 15 \mu M$), a *UGT1A4* specific inhibitor. The promoter region of *UGT1A4* is polymorphic, and compared with those homozygous for the common allele, lower enzymatic activity was observed in microsomes from individuals heterozygous for $-163G < A$, $-219T < G$, and $-217C < T$ ($P = 0.009$, $P = 0.014$, and $P = 0.009$, respectively). These results indicate that variability in glucuronidation could contribute to response to anastrozole in the treatment of breast cancer.

Introduction

Breast cancer is the most frequently diagnosed cancer in women and the second most frequent cause of cancer-related death. In developed countries, around 75% of all breast cancers occur in postmenopausal women, of whom about 80% are estrogen receptor positive (Anderson et al., 2002). Until recently, tamoxifen (TAM) has been the adjuvant treatment of choice for both pre- and postmenopausal women with estrogen receptor-positive early breast cancer (Kamdem et al., 2010). Tumor recurrence and mortality in women with estrogen receptor-positive breast cancer are significantly decreased after 5 years of adjuvant TAM. Nonetheless, yearly recurrence rates remain above 2%, with the Early Breast Cancer Trialists' Collaborative Group study showing a 15-year recurrence of more than 30% (EBCTCG, 2005). In addition, a small proportion of women have serious adverse effects, including increased incidence of endometrial cancer, thromboembolism, and cerebrovascular events (Wysowski et al., 2002; Braithwaite et al., 2003; Cuzick, 2003; EBCTCG, 2005; Lewis, 2007). Thus, alternative hormonal therapies have been sought for adjuvant treatment of breast cancer.

In the past decade, a number of aromatase inhibitors (AIs) have been developed as an alternate approach to TAM for the treatment of

estrogen receptor-positive breast cancer. The current third-generation AIs (anastrozole, exemestane, and letrozole) are highly specific to the aromatase enzyme and have fewer adverse effects than do previous generations of AIs (Fabian, 2007). Anastrozole binds reversibly to the aromatase enzyme and inhibits the conversion of androgens to estrogens in peripheral tissues outside the central nervous system and a few central nervous system sites in various regions in the brain (Simpson, 2003). Evidence from several clinical trials indicates that anastrozole may be superior to TAM as a first-line therapy for postmenopausal women with metastatic breast cancer (Ferretti et al., 2006). Results from at least eight major clinical trials indicate that anastrozole alone is associated with longer disease-free survival than is therapy with TAM alone (Eisen et al., 2008), which supports the use of anastrozole as a first-line therapy or as a second-line therapy after treatment with TAM.

Although anastrozole has demonstrated some superiority relative to TAM (Needleman and Tobias, 2008), many patients still experience a recurrence of breast cancer. In addition, there is substantial inter-individual variability with respect to tolerability, and musculoskeletal complaints can be so severe that some patients withdraw from therapy. This variability is consistent with possible differences among patients in drug pharmacokinetics and/or pharmacodynamics, potentially driven by host genetic variability. These factors, if understood, would offer the potential for individualizing treatment and ensuring that patients receive optimal therapy.

Anastrozole is predominantly modified by hepatic metabolism via oxidation by CYP3A4 into hydroxyl anastrozole, which may further

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ABBREVIATIONS: AIs, aromatase inhibitors; HLM, human liver microsomes; *m/z*, mass-to-charge ratio; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism; TAM, tamoxifen; UDPGA, UDP glucuronide.

Fig. 1. Chemical structures of anastrozole and lamotrigine. Anastrozole, 2,2'-(5-(1H-1,2,4-triazol-1-ylmethyl)-1,3-phenylene)bis(2-methylpropanenitrile) (A) and lamotrigine, 6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine (B) and potential sites for glucuronidation.

undergo glucuronidation by UGT1A4 into hydroxyl anastrozole glucuronide (Dowsett et al., 2001; Kamdem et al., 2010). Anastrozole can also undergo direct glucuronidation catalyzed by UGT1A4 into anastrozole N-glucuronide (Kamdem et al., 2010; Lazarus and Sun, 2010). UGT1A4 is a conjugative detoxification enzyme that facilitates water solubility and excretion of various substrates (Jakoby and Ziegler, 1990). The variability of human glucuronidation activity by UGT1A family is influenced by the presence of single-nucleotide polymorphisms (SNPs), which have been identified not only in the coding regions of the *UGT1A* unique first exons and the common exons 2–5, but also in the 5'-flanking regions (Guillemette et al., 2000a,b; Strassburg et al., 2002; Ehmer et al., 2004; Wiener et al., 2004; Lankisch et al., 2005; Benoit-Biancamano et al., 2009). These SNPs have been shown to alter glucuronidation activity and/or have been associated with the risk of cancer, toxicity, response to therapy,

and unwanted drug adverse effects (Ando et al., 1998; Guillemette et al., 2000a; Vogel et al., 2001; Strassburg et al., 2002; Wiener et al., 2004; Benoit-Biancamano et al., 2009). Therefore, it is conceivable that differences in anastrozole glucuronidation may contribute to the overall variability in treatment effect experienced by patients. Previous studies suggest that UGT1A4 coding SNPs may not contribute to variability in response to anastrozole, but the potential impact of *UGT1A4* promoter SNPs on anastrozole glucuronidation has not been explored. To address this issue, we examined the glucuronidation of anastrozole in human liver microsomes and analyzed the effect of genetic variants of *UGT1A4* on anastrozole glucuronidation.

Materials and Methods

Chemicals and Reagents. Anastrozole (2,2'-(5-(1H-1,2,4-triazol-1-ylmethyl)-1,3-phenylene)bis(2-methylpropanenitrile) was obtained from Toronto Research Chemicals Inc. (Toronto, ON, Canada). Lamotrigine (6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine); hecogenin, alamethicin, magnesium chloride, Tris-HCl, and UDP glucuronic acid (UDPGA) were purchased from Sigma-Aldrich (St Louis, MO). Baculovirus-expressed human UGT1A4 was purchased from BD Gentest Corp. (Woburn, MA). Rabbit antihuman UGT1A4 primary and secondary antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Precast 10% acrylamide gels were obtained from Bio-Rad Laboratories (Hercules, CA). Chemiluminescence reagents were obtained from GE Healthcare (Piscataway, NJ). All other reagents were of high-performance liquid chromatography grade or of the highest grade commercially available.

Human Liver Microsomes (HLMs). The HLMs used for this study have been described previously (Edavana et al., 2012). In brief, human liver specimens ($n = 96$; from noncancerous subjects, collected and frozen 0–3 hours after surgical excision) were obtained from the Cooperative Human Tissue Network. All liver specimens were from white, African American, or race-unknown donors ranging in age from 26 through 102 years, with 54 male, 36 female, and six unknown sex. African Americans were excluded from this study because of low numbers that precluded racial comparisons. All liver specimens were snap-frozen after harvest and were confirmed as histologically normal tissue by the Cooperative Human Tissue Network. Microsomes were prepared from human liver tissue as previously described (Turesky et al., 1991) and stored frozen at -80°C until assayed. Liver microsomal protein levels were

Fig. 2. Mass spectrometry of anastrozole, lamotrigine, and their glucuronides. Representative extracted ion chromatogram of anastrozole (A), lamotrigine (B), and their glucuronides produced by HLM incubations.

Fig. 3. Inhibition of anastrozole glucuronidation by hecogenin. IC_{50} values were calculated in HLM (15 μ M; A) and recombinant UGT1A4 (4.72 μ M; B). Each microsomal incubation with inhibitor was performed in triplicate.

determined using the Bradford method (Bradford, 1976) with bovine serum albumin as a standard. We also included a mixture of liver microsomes pooled from 24 individual donors (BD Gentest Corp.). Pooling was based on cytochrome P450 activity according to manufacturer data sheet.

Quantitative Real-Time Polymerase Chain Reaction. Total RNA from human liver samples was isolated using TRIzol (Invitrogen, Carlsbad, CA) and used as a template for cDNA synthesis with Superscript II (Invitrogen, Grand Island, NY). Quantitative real-time polymerase chain reaction (PCR) was performed using a Prism 7900HT Sequence Detection System and SYBR Green PCR MasterMix (Applied Biosystems, Foster City, CA). The following primers were used: 5'-AGAGAGGTGTCA GTGGTGGATCT-3' (forward) and 5'-AACAGCCACACGGATGCATA-3' (reverse) for *UGT1A4* and 5'-ATCCTGGCCTCGCTGCC-3' (forward) and 5'-CTCCTGCTTGCTGATCCACAT-3' (reverse) for β -actin (reverse). The PCR conditions were as follows: an initial denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 1 minute. Amplified products were monitored by measuring the increase of fluorescence intensity from the SYBR green dye that binds to the double-stranded DNA amplification product. The dissociation curves for each reaction were examined to ensure amplification of a single PCR product in the reaction. The fold changes in mRNA levels were determined after normalizing the gene expression levels by those of β -actin ($2^{-\Delta\Delta C_t}$ method) (Schmittgen and Livak, 2008).

Glucuronidation of Anastrozole and Lamotrigine by HLMs. The glucuronidation of anastrozole and lamotrigine were measured in pooled HLMs, recombinant UGT1A4, and microsomes from 96 individual human livers. Enzymatic assays were performed according to standard procedure (Benoit-Biancamano et al., 2009; Kamdem et al., 2010). Microsomes were preincubated with alamethicin (25 μ g ml^{-1} in final incubation) on ice for 15 minutes. Alamethicin was dissolved in 90/10 incubation buffer/ethanol. The final concentration of ethanol in the reaction was less than 0.1%. Incubations (100 μ l) contained substrate (anastrozole or lamotrigine, 100 μ M), 50 mM Tris-HCl buffer (pH 7.4), 5 mM $MgCl_2$, 100 μ g ml^{-1} microsomes/20 μ g ml^{-1} recombinant UGT1A4, and 5 mM UDPGA and were performed at 37°C for 90 minutes. The reactions were terminated by the addition of 20 μ l acetonitrile, followed by centrifugation at 13,000g for 15 minutes. Aliquots (1 μ l) were then analyzed by liquid chromatography–tandem mass spectrometry. Incubations without UDPGA, anastrozole, lamotrigine,

or microsomes were performed as controls to ensure that produced metabolites were microsome and UDPGA dependent. Metabolite quantitation was performed using an Aquity ultra-performance liquid chromatography (Waters, Milford, MA) interfaced source to an TSQ-QuantumUltra triple quadrupole (ThermoScientific, SanJose, CA) with use of a heat-assisted electrospray ionization source. An Aquity ultra-performance liquid chromatograph bridged ethane-silicon hybrid C18 column was operated with a linear gradient from 5% acetonitrile/0.1% formic acid to 80% acetonitrile/0.1% formic acid in 5 minutes at 0.4 ml/min. Anastrozole and anastrozole glucuronide were acquired by monitoring the ion transition of m/z (mass-to-charge ratio) 294.0 to 225.2 and m/z 470.2 to 225.2, respectively. Lamotrigine and lamotrigine glucuronide were acquired by monitoring the ion transition of m/z 256 to 166.018 and m/z 432 to 345.000, respectively. Because of the absence of authentic standards for anastrozole glucuronide and lamotrigine glucuronide, quantification of the glucuronide was accomplished using a standard curve for anastrozole and lamotrigine.

Inhibition of Anastrozole Glucuronidation. Anastrozole (100 μ M) was incubated in the absence or presence of different concentrations of hecogenin (0–100 μ M), a known UGT1A4 inhibitor (Uchaipichat et al., 2006). Incubations were performed for 90 minutes with use of 100 μ g protein ml^{-1} recombinant UGT1A4 or 0.5 mg protein ml^{-1} of pooled HLM.

Genotyping. Genotyping for *UGT1A4* SNPs was performed in 96 samples. Genotyping for *UGT1A4* 5'-sequences (-36G>A, -163G>A, -217T>G and -219C>T) and exon1 (70C>A and 142 T>G) SNPs were performed as described previously (Saeki et al., 2005; Benoit-Biancamano et al., 2009). All amplification was performed using 1 U of Taq polymerase. Sequencing was performed using an ABI Prism 3700 DNA analyzer (Applied Biosystems).

Statistical Analysis. Both parametric and nonparametric tests were performed to examine the correlation of anastrozole glucuronidation with *UGT1A4* mRNA, lamotrigine glucuronidation, and *UGT1A4* SNPs. In addition, *UGT1A4* mRNA was correlated with *UGT1A4* SNPs. In parametric one-way analysis of variance, non-Gaussian-distributed variables were log transformed and analysis was implemented using Procedure Generalized Linear Model. Nonparametric one-way analysis of variance was executed using Procedure Non-Parametric One Way Analysis of Variance. $P < 0.05$ (2-sided) was considered to be statistically significant, and all analyses were performed using SAS software (version 9.2; Statistical Analysis Systems, Cary, NC).

Results

Analysis of Anastrozole Glucuronidation in HLM. Chemical structures of anastrozole, letrozole, and their glucuronides are presented in Fig. 1. Representative ion chromatograms show that the retention times of anastrozole glucuronide and anastrozole were 2.87 minutes and 3.95 minutes, respectively (Fig. 2A). The retention times of letrozole glucuronide and letrozole were 1.35 minutes and 1.63 minutes, respectively (Fig. 2B). Hecogenin was examined because of its ability to inhibit anastrozole glucuronidation (Wiener et al., 2004). Hecogenin at 5 μ M inhibited anastrozole glucuronidation activity by 40% in pooled HLMs (IC_{50} = 15 μ M) and 65% in recombinant UGT1A4 (IC_{50} = 4.72 μ M). At 50 μ M, hecogenin reduced anastrozole glucuronide formation by 75% in pooled HLMs and 90% in recombinant UGT1A4 (Fig. 3, A and B).

Anastrozole Glucuronidation by HLM. Interindividual variability of anastrozole glucuronidation in 96 samples ranged from 0.023 to 5.4 pmol/mg/min protein. There was a significant correlation between UGT1A4 mRNA level and anastrozole glucuronidation (Fig. 4A). Anastrozole glucuronide formation was highly correlated with letrozole glucuronidation, a known UGT1A4 substrate (Fig. 4B).

Effect of UGT1A4 Genotype on Anastrozole Glucuronidation. To determine the contribution of UGT1A4 coding and promoter polymorphisms to anastrozole glucuronidation, dideoxy sequencing was performed on genomic DNA from liver specimens of 96 human subjects. Previous studies suggested that coding region SNPs 70C>A and 142T>G can alter UGT1A4 catalytic activities (Ehmer et al., 2004; Wiener et al., 2004; Benoit-Biancamano et al., 2009), but no significant association between these SNPs with anastrozole glucuronidation

(Fig. 5A) and UGT1A4 mRNA (Fig. 5B) were found in the current study. We then examined SNPs in the promoter region of UGT1A4. Sequencing analysis of UGT1A4 promoter region revealed the existence of four different SNPs of variable frequencies: [-36G>A (0.07), -163G>A (0.13), -217T>G (0.08), and -219C>T (0.16)]. SNPs -163G<A (P = 0.009), -217T<G (P = 0.009), and -219C<T (P = 0.014) were significantly correlated with anastrozole glucuronidation (Fig. 6). In addition, SNPs -163G<A (P = 0.009), -217T<G (P = 0.009), and -219C<T (P = 0.013) were significantly correlated with UGT1A4 mRNA expression levels. Microsomes from samples heterozygous for the T<G allele at position -217 exhibited significantly increased glucuronidation activity toward anastrozole, compared with microsomes from samples heterozygous for the G<A and C<T at positions -163 and -219, respectively. Except SNPs 142T>G and -163G<A, none of the SNPs are consistent with Hardy Weinberg equilibrium, possibly because of the presence of unknown population substructure. There was no evidence of linkage of the UGT1A4 promoter SNPs with each other or with the coding region SNPs. Thus, the correlations of individual SNPs with UGT1A4 activity were examined separately (Table 1).

Discussion

Aromatase inhibitors have been approved as a first-line treatment option for hormone-dependent advanced breast cancer in postmenopausal patients. However, emerging data suggest high interindividual variability in the beneficial and adverse effects of anastrozole and other aromatase inhibitor drugs (Sanford and Plosker, 2008).

Fig. 4. Correlation plot of anastrozole glucuronidation with UGT1A4 mRNA (A) and letrozole glucuronidation (B). The r and P values specify Spearman's correlation coefficient and statistical significance, respectively.

Fig. 5. Influence of *UGT1A4* coding region SNPs on anastrozole glucuronidation (A) and *UGT1A4* mRNA expression levels (B). The (+) sign and line inside each box indicate mean and median, and the upper and lower limits of the box are 75th and 25th percentiles, respectively. The vertical bars above and below represent the maximum and minimum values, respectively. The solid circles outside the box are outlier values. *P* values indicate the statistical significance of Kruskal Wallis tests (nonparametric one-way analysis of variance).

Anastrozole is metabolized and cleared mainly through *N*-dealkylation, hydroxylation via the CYP3A4 enzyme (Dowsett et al., 2001; Antoniou and Tseng, 2005) and glucuronidation via the UGT1A4 enzyme (Kamdern et al., 2010). The major metabolites of anastrozole identified in urine and plasma are triazole, OH-anastrozole, the glucuronide of OH-anastrozole, and the *N*-glucuronide of anastrozole [Astra-Zeneca, 2007 (www1.astrazeneca-us.com/pi/arimidex.pdf, 2006)]. *N*-glucuronidation of anastrozole is catalyzed predominantly by UGT1A4 (and to a lesser extent by UGT2B7 and UGT1A3) (Kamdern et al., 2010; Lazarus and Sun, 2010), resulting in deactivation and elimination of the active drug. Therefore, it is possible that variability in anastrozole glucuronidation because of genetic factors may contribute to the overall interindividual variability in efficacy and tolerability experienced by patients.

In this study, recombinant UGT1A4 glucuronidated anastrozole, consistent with other studies (Kamdern et al., 2010; Lazarus and Sun, 2010). However, UGT1A4 may not be the only hepatic UGT participating in the glucuronidation of anastrozole. UGT2B7 and UGT1A3 present in HLM may also have a minor role in anastrozole glucuronidation (Kamdern et al., 2010; Lazarus and Sun, 2010). Moreover, UGT1A4 is expressed in

the liver, with extensive interindividual variability mostly dictated by environmental factors, age, sex, race, genetic variability, and concomitant drug administration (Wilkinson, 2005).

Previous studies have demonstrated that hecogenin is a specific UGT1A4 inhibitor (Uchaipichat et al., 2006; Kamdern et al., 2010). In the current study, hecogenin significantly inhibited anastrozole glucuronidation in both HLM ($IC_{50} = 15 \mu M$) and recombinant UGT1A4 ($IC_{50} = 4.72 \mu M$). These results were consistent with those of Kamdern et al. (2010), but not with Lazarus and Sun (2010), and Erickson et al. (2012), where clozapine (a substrate of UGT1A4) glucuronidation was inhibited by 90% with 10 μM hecogenin. Interindividual variability of UGT1A4, and possibly a minor role of UGT1A3 and UGT2B7 in anastrozole glucuronidation may have an effect on inhibition studies. Variations in substrate concentration and purity may also have an effect on experimental results. Moreover, Zhou et al. (2010) suggest that multiple aglycone binding sites exist in UGT1A4, which may result in atypical kinetics (both homotropic and heterotropic) in a substrate-dependent fashion.

Anastrozole glucuronidation was highly correlated with lamotrigine glucuronidation (a diagnostic substrate for UGT1A4; $r = 0.99$, $P < 0.0001$),

Fig. 6. Influence of *UGT1A4* promoter SNPs on anastrozole glucuronidation activity in HLM. The (+) sign and line inside each box indicate mean and median, and the upper and lower limits of the box are 75th and 25th percentiles, respectively. The vertical bars above and below designate the maximum and minimum values, respectively. The solid circles outside the box are outlier values. *P* values indicate the statistical significance of rank sum tests (nonparametric two-sample *t* test).

but some recent studies suggest that lamotrigine may not be entirely specific for *UGT1A4* (Erickson-Ridout et al., 2011). However, these results support the predominant (but not the sole) role of *UGT1A4* in the direct glucuronidation of anastrozole.

UGT1A4 is expressed primarily in the liver, but it is also expressed in biliary tissue, colon, and breast (Strassburg et al., 1997; Chouinard et al., 2006). Glucuronidation activities in different human tissues have been shown to exhibit a high degree of interindividual variation that cannot be explained by unique tissue specificity of *UGT1A4* gene expression (Strassburg et al., 2000; Shipkova et al., 2001). A potential contributor to variability is the presence of SNPs in the promoter and coding regions of the *UGT1A4* gene, leading to quantitative or qualitative alterations of specific catalytic activities among individuals. Our data on *UGT1A4* mRNA expression levels in 96 human subjects showed marked interindividual variability, suggesting that genetic variants in *UGT1A4* could impact anastrozole pharmacogenomics. We examined the association between *UGT1A4* promoter and coding SNPs and variability in anastrozole glucuronidation. Previous

UGT1A4 genotype-phenotype correlations have focused primarily on the coding region SNPs (*UGT1A4* 70C>A and 142 T>G). Consistent with previous studies (Kamdern et al., 2010; Lazarus and Sun, 2010), we found no statistically significant association between these genetic variants and anastrozole glucuronidation formation rates. However, there are reports of an association of coding region SNPs with differential metabolic activity toward mutagenic amines and endogenous steroids, altering hepatic metabolism and detoxification (Ehmer et al., 2004; Benoit-Biancamano et al., 2009; Gulcebi et al., 2011; Zhou et al., 2011). More recently, functional SNPs in the promoter elements located in the 5'-upstream region of *UGT1A4* have been described (Saeki et al., 2005; Erichsen et al., 2008; Benoit-Biancamano et al., 2009; Menard et al., 2009). *UGT1A4* expression and induction were reduced in the presence of SNPs at positions -219 and -163 (Erichsen et al., 2008). We observed a similar decrease in anastrozole glucuronidation and *UGT* mRNA expression levels in liver microsomes from individuals heterozygous for -163G<A and -219C<T alleles. On the other hand, microsomes from individuals heterozygous

TABLE 1
Analysis of anastrozole glucuronidation and UGT mRNA expression levels by *UGT1A4* variants

Variant	Frequency %	Anastrozole glucuronidation median (S.D.) pmol/ μ g/min	UGT1A4 mRNA median (S.D.)	P for Hardy-Weinberg Equilibrium
70C>A				0.0007
CC	48 (18.7)	0.4 (0.6)	1.4 (1.2)	
AC	26 (28.6)	0.3 (1.7)	1.3 (0.5)	
AA	17 (52.7)	0.8 (1.3)	1.4 (0.3)	
142T>G				0.12
TT	38 (41.8)	0.5 (0.7)	1.4 (0.2)	
TG	36 (39.6)	0.3 (1.4)	1.2 (0.5)	
GG	17 (18.7)	0.3 (1.5)	1.2 (0.4)	
-36G>A				<0.0001
GG	81 (89)	0.3 (1.2)	1.4 (1.4)	
AG	7 (7.7)	1.0 (1.4)	1.5 (0.3)	
AA	3 (3.3)	1.3 (0.6)	1.5 (0.2)	
-163G>A				0.014
GG	73 (79.3)	0.4 (1.2)	1.4 (0.3)	
AG	15 (16.3)	0.7 (1.5)	1.1 (0.5)	
AA	4 (4.3)	0.8 (0.3)	1.4 (0.1)	
-217T>G				0.0002
TT	80 (87.9)	0.3 (1.1)	1.3 (0.3)	
TG	8 (8.8)	1.5 (1.7)	1.7 (0.3)	
GG	3 (3.3)	1.9 (1.2)	1.7 (0.3)	
-219C>T				0.03
CC	69 (74.2)	0.4 (1.1)	1.4 (0.3)	
CT	19 (20.4)	0.9 (0.7)	1.0 (0.4)	
TT	5 (5.4)	1.0 (2.2)	1.2 (0.6)	

for the -217T<G allele exhibited significantly higher glucuronidation activity toward anastrozole and increased UGT1A4 transcriptional activity. These promoter SNPs (-163G<A, -217 T<G and -219C<T) were significantly associated with both anastrozole glucuronidation and UGT1A4 mRNA expression levels. Benoit-Biancamano et al., reported a strong linkage disequilibrium of promoter SNPs (-163A, -219T, -419A, and -463T) with coding region variant 142 T, but in our study, none of these UGT1A4 promoter SNPs were in linkage disequilibrium either with each other or with the coding region SNPs (Benoit-Biancamano et al., 2009). Examination of SNP haplotypes in relation to enzymatic activity would be optimal, but our sample size was insufficient to examine the effect of multiple SNPs in a statistically robust manner.

Studies have demonstrated a consistent association of *UGT1A4* genotype with cancer risk, toxicities, and drug response (Innocenti et al., 2001; Nakajima and Yokoi, 2005; Figueroa et al., 2008; Murdter et al., 2011). Improvement of the genetic model predictive of UGT1A4 phenotype could provide a biomarker for predicting cancer risk and therapeutic efficacy. To our knowledge, this study describes for the first time the association of *UGT1A4* promoter SNPs and anastrozole glucuronidation. Limitations to the study include the unavailability of authentic standards to examine the isomeric selectivity of anastrozole glucuronidation and, thus, stereospecific genotype-phenotype analyses. Nonetheless, we have demonstrated a significant impact of UGT1A4 genotype on anastrozole glucuronidation, which highlights the importance of UGT1A4 genotype in anastrozole pharmacogenomics studies.

Authorship Contributions

Participated in research design: Edavana, Kadlubar.

Conducted experiments: Edavana, Williams.

Contributed new reagents or analytic tools: Boysen.

Performed data analysis: Edavana, Dhakal.

Wrote or contributed to the writing of the manuscript: Edavana, Kadlubar, Penney, Yao-Borengasser, Boysen.

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