Sulfation of 4-Hydroxy Toremifene: Individual Variability, Isoform Specificity, and Contribution to Toremifene Pharmacogenomics

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ABSTRACT:

Toremifene (TOR) is a selective estrogen receptor modulator used in adjuvant therapy for breast cancer and in clinical trials for prostate cancer prevention. The chemical structure of TOR differs from that of tamoxifen (TAM) by the presence of a chlorine atom in the ethyl side chain, resulting in a more favorable toxicity spectrum with TOR. In addition, some patients who fail on TAM therapy benefit from high-dose TOR therapy. Several studies have indicated that functional genetic variants in the TAM metabolic pathway influence response to therapy, but pharmacogenomic studies of patients treated with TOR are lacking. In this study, we examined individual variability in sulfation of 4-hydroxy TOR (4-OH TOR) (the active metabolite of TOR) in human liver cytosols from 104 subjects and found approximately 30-fold variation in activity. 4-OH TOR sul-

fation was significantly correlated ($r = 0.98$, $P < 0.0001$) with β -naphthol sulfation (diagnostic for SULT1A1) but not with 17 β estradiol sulfation, a diagnostic substrate for SULT1E1 ($r = 0.09$, $P = 0.34$). Examination of recombinant sulfotransferases (SULTs) revealed that SULT1A1 and SULT1E1 catalyzed 4-OH TOR sulfation, with apparent K_m values of 2.6 and 6.4 μM and $V_{\max}$ values of 8.5 and 5.5 $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$, respectively. 4-OH TOR sulfation was inhibited by 2,6-dichloro-4-nitrophenol ($\text{IC}_{50} = 2.34 \pm 0.19 \mu\text{M}$), a specific inhibitor of SULT1A1. There was also a significant association between *SULT1A1* genotypes and copy number and 4-OH TOR sulfation in human liver cytosols. These results indicate that variability in sulfation could contribute to response to TOR in the treatment of breast and prostate cancer.

Introduction

Tamoxifen (TAM), approved for pharmaceutical use in the United States in 1977, has proven value in the treatment of patients with hormone receptor-positive breast cancer. In early studies, the use of adjuvant TAM therapy for 5 years demonstrated benefit in terms of both improved disease-free survival and overall survival. Unfortunately, the use of TAM has also been shown to increase the risk of thromboembolic events, ocular changes, and endometrial carcinoma. Thus, alternative hormonal therapies have been sought for adjuvant treatment of breast cancer.

Toremifene (TOR) is a nonsteroidal triphenylethylene selective estrogen receptor modulator that binds to estrogen receptors, exerting either estrogenic or antiestrogenic effects, depending on the end organ, dose, and duration of therapy (Kallio, 1986). TOR varies from TAM in a single chloride ion addition on a side chain, which alters the metabolic pathway compared with TAM and results in a more favorable toxicity profile, at least in animal studies (Hirsimäki et al., 2002). TOR has been demonstrated to inhibit cell proliferation in both breast and endometrial tissues (Gershanovich et al., 1997) and acts as an

anti-estrogen in breast tissue, inducing apoptosis and inhibiting cells from entering mitosis in human breast cancer cells (Huovinen et al., 1993).

Findings from preclinical studies, as well as early clinical experience, raised concerns regarding long-term effects of TAM use. In animal studies, TAM has been shown to be far more genotoxic and carcinogenic than TOR because of increased DNA adduct formation and induction of hepatic and endometrial cancers in the rat (White, 1999; Hirsimäki et al., 2002). Phase 2 trials of TAM and TOR in patients with metastatic breast cancer confirmed the safety and efficacy of TOR (Valavaara, 1990; Hayes et al., 1995). In addition to findings with breast cancer, TOR has been associated with a decrease in the incidence of high-grade prostatic intraepithelial neoplasia and prostate cancer and an increased probability of survival in the transgenic adenocarcinoma of the mouse prostate mouse model (Raghow et al., 2002).

TOR undergoes metabolism by multiple cytochromes P450 to produce the active metabolite, 4-hydroxy TOR (4-OH TOR) (Berthou et al., 1994). Subsequently, UDP-glucuronosyltransferase and sulfotransferases (SULTs) participate in 4-OH TOR metabolism (Crewe et al., 2002; Kim et al., 2003). SULTs, along with UDP-glucuronosyltransferases, are Phase II detoxification enzymes whose physiological function is to increase the water solubility of various substrates, thus facilitating their excretion (Jakoby and Ziegler, 1990). For this reason, factors influencing phase II enzymatic

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ABBREVIATIONS: TAM, tamoxifen; SULT, sulfotransferases; TOR, toremifene; 4-OH TOR, 4-hydroxy toremifene; PAPS, 3'-phosphoadenosine 5'-phosphosulfate; PCR, polymerase chain reaction; DMSO, dimethyl sulfoxide; TBAHS, tetrabutylammonium hydrogen sulfate; DCNP, 2,6-dichloro-4-nitrophenol; UTR, untranslated region; SNP, single-nucleotide polymorphism; ANOVA, analysis of variance.

TABLE 1
Kinetics of 4-OH TOR sulfation

	K_m	V_{max}	V_{max}/K_m^a
	μM	$nmol \cdot min^{-1} \cdot mg \text{ protein}^{-1}$	
SULT1A1	2.6 ± 0.4	8.5 ± 0.3	3.3 ± 0.5
SULT1E1	6.4 ± 0.09	5.5 ± 0.2	0.9 ± 0.04
Human liver cytosol	3.5 ± 0.05	1.4 ± 1.03	0.4 ± 0.3

^a Data are mean $\pm$ S.D. of triplicate measurements.

activity could exert a significant effect on both toxicity and therapeutic response to drugs that are their substrates. These enzyme families are polymorphic in nature, and many studies have demonstrated that genetic variants in drug-metabolizing enzymes can influence both toxicity and response to therapy. Although the phase I metabolism of TOR has been fairly well described, specific SULTs responsible for 4-OH TOR sulfation have not been explored. To address this, we examined the sulfation of 4-OH TOR in human liver cytosols and recombinant SULTs and examined the effect of genetic variants on 4-OH TOR sulfation.

Materials and Methods

Materials. TOR and 4-OH TOR were provided by Orion Pharma (Helsinki, Finland). 3'-Phosphoadenosine 5'-phosphosulfate (PAPS; estimated purity 97%, determined by sequential thin-layer chromatography) was obtained from University of Dayton Chemistry Department (Dayton, OH). Sequencing and polymerase chain reaction (PCR) primers were purchased from Invitrogen (Carlsbad, CA). [³⁵S]Phosphoadenosine phosphosulfate (specific activity 2.2 Ci/mmol) was purchased from PerkinElmer Life and Analytical Sciences (Waltham, MA). All other chemicals used were of reagent grade from Thermo Fisher Scientific (Waltham, MA).

Sulfation by Human Liver Cytosols. Human liver specimens ($n = 104$) were obtained from the Cooperative Human Tissue Network. All liver specimens were from white donors ranging in age from 10 to 85 years, with 56 male and 44 female donors. African Americans were excluded from this study because of low numbers that precluded racial comparisons. All liver specimens were snap-frozen upon harvest and were confirmed as histologically normal tissue by the Cooperative Human Tissue Network. Tissue specimens that exhibited abnormalities were excluded from this study. Cytosols were prepared from human liver tissue as described previously (King et al., 2000) and were stored frozen at $-80^\circ C$ until assayed. Cytosolic protein levels were determined using the Bradford (1976) method with bovine serum albumin as a standard.

A

*Substrate concentration used in these assays ranges from 0.1 μM –30 μM –30

B

*Substrate concentration used in these assays ranges from 0.1 μM –30 μM –30

FIG. 1. 4-OH TOR sulfation kinetic analysis. Michaelis-Menten and Eadie-Hofstee plots of 4-OH TOR sulfation by SULT1A1 (A) and SULT1E1 (B) with concentrations of 4-OH TOR ranging from 0.1 to 30 μM and 20 μM [³⁵S]PAPS.

Enzymatic activity analyses were performed using a modification of a previously reported protocol for 4-hydroxy TAM sulfation determination (Chen et al., 2002). Incubations to determine activity toward 4-OH TOR contained 100 μ M 4-OH TOR (dissolved in dimethyl sulfoxide (DMSO)/H₂O, 1:3), 50 mM potassium phosphate buffer, pH 6.2, 20 μ M [³⁵S]PAPS, and 100 μ g of cytosolic protein in a final volume of 100 μ L. Initial assays were performed to determine optimal pH, and we found that compared with pH 7.8, enzymatic activity was highest at pH 6.2. Therefore, this pH was used for all subsequent experiments. The final DMSO concentration in the reactions was <0.1%. Control reactions were run without substrate but with the appropriate volume of the DMSO vehicle. Reactions were incubated for 15 min at 37°C and then terminated by adding 50 μ L acetonitrile/acetic acid (96:4) followed by analysis using a Waters Alliance high-performance liquid chromatography system (Waters, Milford, MA) instrument attached to a Radiomatic model 150TR flow scintillation analyzer and pump control module (PerkinElmer Life and Analytical Sciences). The reaction products were separated using a Waters Symmetry C18 column (4.6 $\times$ 150 mm, 10 μ m) (Waters) and eluted at a flow rate of 1.0 ml/min with a gradient of 70% 2 mM tetrabutylammonium hydrogen sulfate (TBAHS) and 30% acetonitrile for 35 min, 55% 2 mM TBAHS and 45% acetonitrile for 15 min, followed by 2 mM TBAHS and 30% acetonitrile for 20 min. The injection volume was 100 μ L, and the retention times were 12.1 and 45.2 min for SO₄-TOR and 4-OH TOR, respectively. Activity toward β -naphthol was determined using a colorimetric assay as described previously (Frame et al., 2000). Activity toward 17 β -estradiol used radiolabeled E₂, the sulfate acceptor cosubstrate, rather than radioactively labeled PAPS (Falany et al., 1995).

Sulfation by Recombinant SULTs. Sulfation activity was determined using 4-OH TOR as substrate with each of nine different bacterially expressed human SULT isoforms. All SULTs were expressed in *Escherichia coli* using the pET vector (Invitrogen) to generate the native form of the enzyme and then were purified by DEAE-Sepharose chromatography to obtain a preparation suitable for enzymatic characterization (Falany et al., 1995). The resulting preparations were approximately 80% pure, and activities were calculated on the basis of total protein. Assays were performed with each of the expressed human SULTs (SULT2A1, SULT1E1, SULT2B1a, SULT2B1b, SULT1A1, SULT1A3, SULT1B1, SULT1C1, and SULT1C2) at 4-OH TOR concentrations ranging from 0.1 to 30 μ M. 4-OH TOR and its sulfated metabolite were analyzed using Waters Alliance high-performance liquid chromatography system connected to a 2996 photodiode array detector and Radiomatic model 150TR flow scintillation analyzer (PerkinElmer Life and Analytical Sciences).

Kinetics of 4-OH TOR Sulfation. For determination of apparent K_m values, reactions were monitored for linearity with respect to both time and protein concentration. Because cytosolic SULTs frequently display substrate inhibition with high-affinity substrates, experiments to establish kinetic parameters were run at low substrate concentrations in the linear range to minimize the effects of substrate inhibition (Zhang et al., 1998). The reaction mixture contained 50 mM potassium phosphate, pH 6.2, 20 μ M PAPS, 100 μ g/ml SULT1A1, and varying concentrations of 4-OH TOR (0.1–30 μ M) in a final volume of 100 μ L. Reactions were initiated by the addition of enzyme to the reaction mixture and then incubation for 15 min at 37°C. The reactions were terminated by precipitation of the enzyme by adding 50 μ L of acetonitrile/acetic acid (96:4). Assays were performed in triplicate and corrected for background activity using a control with no substrate added. Kinetic constants were calculated by fitting with the Michaelis-Menten equation using the Enzyme Kinetics Module of Sigma-Plot 11 (Systat Software, San Jose, CA).

Chemical Inhibition of SULT1A1 Activities. 2,6-Dichloro-4-nitrophenol (DCNP) is a selective inhibitor of SULT1A1 (Weinshilboum, 1986). Inhibition experiments were performed using a pool of human liver cytosols. DCNP was dissolved in ethanol. The final concentrations of DCNP in the assay ranged from 0.1 to 10 μ M. After an incubation period (15 min), the reactions were halted using 50 μ L of acetonitrile/acetic acid (96:4). Formation of SO₄-TOR was quantified as described for sulfation of recombinant enzymes.

SULT1A1 Genotyping. Genotyping for *SULT1A1**1/2 and 3'-untranslated region (UTR) single-nucleotide polymorphisms (SNPs) was performed as described previously (Yu et al., 2010). Genotype was determined by direct sequencing using the CEQ Dye Terminator Cycle Sequencing Quick Start

Sequencing Kit and the CEQ 8800 Genetic Analysis System (Beckman Coulter, Inc., Fullerton, CA).

SULT1A1 Copy Number Variation Assay. *SULT1A1* copy number determination was performed by real-time PCR in an ABI PRISM Sequence Detection System 7900 Instrument using the TaqMan Gene Expression Absolute Quantification Assay (Applied Biosystems, Foster City, CA). A pair of unlabeled PCR primers, 5'-TGCCCGCAACGCAAA-3' and 5'-GGCCATGTGGTAGAAGTGGTAGT-3', and a 5-carboxyfluorescein dye-labeled TaqMan MGB probe, 5'-ATGTGGCAGTTTCC-3', were designed to specifically amplify *SULT1A1*. VIC dye-labeled TaqMan RNaseP, which has two copies per haploid human genome, was used as a control. Amplification was initiated within 10 min at 95°C, followed by 40 amplification cycles (15 s of denaturation at 95°C and 60 s of annealing/extension at 60°C). Each sample was examined in quadruplicate, and copy number was determined using Copycaller software (Applied Biosystems).

Statistical Analysis. Both parametric and nonparametric tests were performed to examine the correlation between 4-OH TOR sulfation, β -naphthol sulfation, 17 β -estradiol sulfation, *SULT1A1* SNPs, and *SULT1A1* copy number as appropriate. In parametric one-way analysis of variance (ANOVA), non-Gaussian distributed variables were log transformed, and analysis was implemented using PROC GLM (SAS Institute, Cary, NC). A *P* value of less than 0.05 (two-sided) was considered to be statistically significant, and all analyses were performed using SAS software (version 9.2; SAS Institute).

Results

Sulfation of 4-OH TOR by Expressed Human SULTs. The structure of 4-OH TOR suggests that one or more of the human SULTs involved in phenol or estrogen conjugation may readily sulfate 4-OH TOR. Therefore, the ability of nine expressed isoforms of human cytosolic SULTs to conjugate 4-OH TOR was investigated. Among the nine SULT isoforms tested, only SULT1A1 and SULT1E1 were capable of conjugating 4-OH TOR. All bacterially expressed SULTs exhibited high activity toward their diagnostic substrates (data not shown). Table 1 shows the apparent K_m values for 4-OH TOR sulfation determined with SULT1A1 and SULT1E1 iso-

FIG. 2. Distribution of 4-OH TOR sulfation activity in human liver cytosols. Interindividual variability in 4-OH TOR sulfation was evaluated in liver cytosols collected from 104 individuals. These reactions were performed as described under *Materials and Methods*. This histogram demonstrates the distribution of 4-OH TOR sulfate ranging from 0.003 to 2.59 nmol $\cdot$ min⁻¹ $\cdot$ mg⁻¹ (0.91 $\pm$ 0.47, *n* = 104). Each value is the average of triplicate incubations.

FIG. 3. Correlation plot of 4-OH TOR sulfation diagnostic substrates for SULT1A1 and SULT1E1 and inhibition by DCNP. A, the correlation between β -naphthol sulfation and 4-OH TOR sulfation was statistically significant ($r = 0.98$, $P < 0.0001$). B, there was no statistically significant association found between 4-OH TOR sulfation and 17 β -estradiol sulfation ($r = 0.09$, $P = 0.34$). C, inhibition of 4-OH TOR sulfation by DCNP, a SULT1A1 inhibitor. Activity was analyzed, and IC_{50} values were calculated ($2.63 \pm 0.19 \mu\text{M}$). Incubations were performed with human liver cytosols, as described under *Materials and Methods*. Each value is the average of three separate determinations.

forms. At 4-OH TOR concentrations up to $30 \mu\text{M}$, substrate inhibition was not observed with either isoform. SULT1A1 had the lowest K_m ($2.6 \mu\text{M}$) (Fig. 1A) for 4-OH TOR sulfation, whereas SULT1E1 had the highest K_m ($6.4 \mu\text{M}$) (Fig. 1B). SULT1A1 had the highest $V_{\max}$ values ($8.5 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$) compared with SULT1E1 ($5.5 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$). The kinetics of sulfation was determined by the ratio of $V_{\max}/K_m$.

Sulfation of 4-OH TOR by Human Liver Cytosols. Sulfation is recognized as an important reaction in the metabolism of 4-hydroxy TAM (Chen et al., 2002; Nowell et al., 2002). However, little is known concerning the sulfation of 4-OH TOR in human tissues. Therefore, the ability of human liver cytosols to catalyze the sulfation of 4-OH TOR was examined. Figure 2 shows the interindividual variability in sulfation of 4-OH TOR by 104 human liver cytosols. 4-OH TOR sulfation was undetectable in some instances and, when detected, ranged from 0.0003 to $2.59 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$. 4-OH TOR sulfation and β -naphthol sulfation (diagnostic substrate for SULT1A1) were highly correlated ($r = 0.98$, $P < 0.0001$) (Fig. 3A). 4-OH TOR sulfation was not correlated with the sulfation of 17 β -estradiol, a diagnostic substrate for SULT1E1 ($r = 0.09$, $P = 0.34$) (Fig. 3B). In addition, DCNP, a specific inhibitor of SULT1A1 activity, was a potent inhibitor of 4-OH TOR sulfation ($IC_{50} = 2.34 \mu\text{M} \pm 0.19$). Approximately 95% of 4-OH TOR sulfation was inhibited by $7 \mu\text{M}$ DCNP (Fig. 3C).

Association of SULT1A1 Genotype and Copy Number with 4-OH TOR Sulfation. Previous studies of SULT1A1 genotype-phenotype correlation have focused primarily on the SULT1A1*1/2 (A213H). We have described functional SNPs in the 3'-UTR of SULT1A1 that are in linkage disequilibrium with SULT1A1*1/2 (Yu et al., 2010). To further determine whether variations in SULT1A1 copy number, 3'-UTR, and SULT1A1*1/2 SNPs were associated with 4-OH TOR sulfation, SULT1A1 copy number, SNPs, and SULT1A1 phenotype were determined in 104 liver samples. The effect of genotype, both alone and in combination with copy number, on enzymatic activity was then determined. When the SULT1A1*1/2 SNP was considered alone, there was a significant association ($P_{\text{ANOVA}} = 0.024$) between genotype and 4-OH TOR sulfation (Fig. 4). We then

examined the effect of SULT1A1 copy number on SULT1A1 enzymatic activity toward 4-OH TOR. As shown in Fig. 5A, there was a significant influence of copy number on enzymatic activity ($P_{\text{ANOVA}} < 0.0001$), with increasing activity with increasing copies of SULT1A1. Haplotypes of the 3'-UTR were constructed as described previously (Ning et al., 2005), and their influence on SULT1A1 enzymatic activity was determined. There was a significant trend associated with different SULT1A1 haplotypes and SULT1A1 activity ($P_{\text{trend}} = 0.008$) (Fig. 5B). We then examined the combined effect of SULT1A1 haplotypes and copy number on 4-OH TOR sulfation. Even when stratified by copy number, the effect of the 3'-UTR haplotypes on SULT1A1 activity was still evident (Fig. 5C). When constructing haplotypes from SNPs, we initially included the SULT1A1*1/2 SNP in the analysis. As in our previous study (Yu et al.,

FIG. 4. Influence of SULT1A1*1/2 genotype on 4-OH TOR sulfation activity in human liver cytosols. Genotyping and enzymatic assays were performed using the procedures described under *Materials and Methods*. Genotype-phenotype relationships were assessed by ANOVA with phenotype as the dependent variable.

FIG. 5. Influence of *SULT1A1* 3'-UTR haplotypes and copy number on 4-OH TOR sulfation. Haplotype/copy number/ phenotype relationships were assessed by ANOVA with phenotype as the dependent variable. There was a significant trend associated with *SULT1A1* 3'-UTR haplotypes and 4-OH TOR sulfation ($P_{trend} = 0.008$) (A), and there was a significant influence of copy number on enzymatic activity ($P_{anova} < 0.0001$) (B). Combined effect of *SULT1A1* haplotypes and copy number on 4-OH TOR sulfation showed a significant effect of 3'-UTR haplotypes on *SULT1A1* activity when stratified by copy number ($P_{trend} = 0.11$ and $P_{diff} > 0.05$) (C).

2010), we found that inclusion of this SNP had no effect on the statistical model (data not shown).

Discussion

TAM therapy has been associated with increased incidence of endometrial carcinoma (Williams-Brown et al., 2011), and the mechanism is thought to involve the formation of TAM-DNA adducts via O-sulfonation of α -hydroxylated TAM metabolites by hydroxysteroid sulfotransferases (Shibutani et al., 1998a,b). However, subsequent animal studies found substantially less TOR adducts compared with TAM, and no hepatocarcinoma was promoted in rats treated with TOR (White et al., 1992; Hard et al., 1993). Because clinical efficacy of TOR for breast cancer patients is similar to that of TAM (Buzdar and Hortobagyi, 1998), the use of TOR, instead of TAM, could reduce the risk of developing endometrial cancer in breast cancer patients (Shibutani et al., 2001). In addition, TOR is under investigation as an adjuvant therapy for prostate cancer; thus, pharmacogenomic studies could predict patients most likely to benefit from TOR. Although phase I metabolism of TOR and genetic variants in those enzymes is likely to influence TOR efficacy, in this study we focused on sulfation of 4-OH TOR, the active metabolite of TOR.

SULT isoforms display a distinct pattern of tissue distribution, and the identification of isoforms involved in the sulfation of 4-OH TOR is required for a better understanding of its pharmacogenomics. SULT isoforms are abundant in the gastrointestinal tract and liver, where they play an important role in drug and xenobiotic sulfation after absorption of compounds from the gastrointestinal tract. In this study, we identified the two major SULT isoforms (*SULT1A1* and *SULT1E1*) involved in the sulfation of 4-OH TOR. The K_m values for *SULT1A1* and *SULT1E1* were 2.6 and 6.4 μ M, respectively.

Our correlation analysis of 4-OH TOR sulfation showed significant association with β -naphthol sulfation, but not with 17 β -estradiol sulfation, and DCNP could potentially inhibit 4-OH TOR sulfation in human liver cytosols. Taken together, these results suggest that *SULT1A1* is the primary hepatic SULT involved in 4-OH TOR

sulfation. Because *SULT1A1* is the most highly expressed hepatic SULT, these findings are expected. *SULT1E1* expression is low in the liver but can be highly expressed in extrahepatic tissues, including breast and prostate epithelia, where it could significantly contribute to 4-OH TOR disposition. Genetic variants in *SULT1E1* could affect TOR pharmacogenomics, but to date the identified SNPs in this gene generally have a low allelic frequency, and in this study, we were unable to examine them because of small sample size. For this reason, we focused our attention on *SULT1A1* genetic variants and their relationship with 4-OH TOR sulfation in vitro.

When analyzed independently, the *SULT1A1**1/2 SNP, *SULT1A1* copy number, and haplotypes constructed from the 3'-UTR SNPs were all significantly associated with 4-OH TOR sulfation. *SULT1A1* copy number accounted for most of the interindividual variability (20.3%), followed by 3'-UTR SNP haplotype (8.0%). The *SULT1A1**1/2 SNP was significantly associated with activity, and 6% of the variability in 4-OH TOR sulfation was attributed to this SNP. When haplotype construction included the *SULT1A1**1/2 SNP, we found that addition of this SNP did not influence the haplotype model. Haplotype GTA is associated with low enzymatic activity; when *SULT1A1**1/2 was included to produce haplotypes GTAG and GTAA, both remained associated with low enzymatic activity, although the ending "G" is considered a high-activity allele when *SULT1A1**1/2 is analyzed alone. This is consistent with our previous study, and the association of *SULT1A1**1/2 found in numerous studies is likely due to the high degree of linkage (Lewontin's $D' = 0.83$) between *SULT1A1**1/2 and the 3'-UTR SNPs (Yu et al., 2010).

The moderate influence of *SULT1A1* genetic variation on 4-OH TOR phenotype suggests the presence of other genetic variants that play a role in modulating *SULT1A1* activity and/or modulation by environmental and dietary factors. Copy number explains the largest proportion of the variation, but addition of the 3'-UTR SNPs improved the accuracy of this model. In this way, functional analysis of other SNPs could lead to a better predictive model for *SULT1A1* phenotype and increase confidence in the reliability of genetic pre-

dictors of individual response to therapeutic agents that are substrates of SULT1A1. Molecular epidemiology studies have consistently shown an association of *SULT1A1* genotype with risk of several cancer types; improvement of the genetic model predictive of SULT1A1 phenotype could reveal an even more substantial contribution of this gene to both cancer risk and therapeutic efficacy.

In summary, hepatic 4-OH TOR sulfation is mediated predominantly by SULT1A1. *SULT1A1**1/2 genotype, copy number, and 3'-UTR SNP haplotypes had a significant association with 4-OH TOR sulfation. Future pharmacogenomic studies of toremifene SULT1A1 should include examination of *SULT1A1* and, when possible, *SULT1E1* genetic variants.

Authorship Contributions

Participated in research design: Edavana, Yu, and Kadlubar.

Conducted experiments: Edavana, Yu, and Williams.

Performed data analysis: Dhakal.

Wrote or contributed to the writing of the manuscript: Edavana and Kadlubar.

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