Comprehensive assessment of cytochromes P450 and transporter genetics with endoxifen concentration during tamoxifen treatment

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Abstract

Objectives—Tamoxifen bioactivation to endoxifen is primarily mediated by CYP2D6; however, substantial variability remains unexplained. Our objective was to conduct a comprehensive assessment of the effect of genetic variation in tamoxifen-relevant enzymes and transporters on steady-state endoxifen concentrations.

Methods—Comprehensive genotyping of CYP enzymes and transporters was conducted using the iPLEX® ADME PGx Pro Panel in 302 tamoxifen treated breast cancer patients. Predicted activity phenotype for 19 enzymes and transporters were analyzed for univariate association with endoxifen concentration, and then adjusted for *CYP2D6* and clinical covariates.

Results—In univariate analysis, higher activity of CYP2C8 (regression $\beta=0.22$, $p=0.020$) and CYP2C9 ($\beta=0.20$, $p=0.04$), lower body weight ($\beta=-0.014$, $p<0.0001$), and endoxifen measurement during winter (each $\beta<-0.39$, $p=0.002$), were associated with higher endoxifen concentration. After adjustment for CYP2D6 diplotype, weight and season, CYP2C9 remained significantly associated with higher concentration ($p=0.02$), but only increased the overall model R-squared by 1.3%.

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Conflict of Interest

None declared

Supplemental Digital Content is available at *Pharmacogenetics and Genomics'* website.

Conclusions—Our results further support a minor contribution of CYP2C9 genetic variability on steady state endoxifen concentration. Integration of clinician and genetic variables into individualized tamoxifen dosing algorithms would marginally improve their accuracy, and potentially enhance tamoxifen treatment outcomes.

Keywords

tamoxifen; endoxifen; pharmacogenomics; CYP2C9; CYP2D6; season

Introduction

Tamoxifen, a selective estrogen receptor modulator, is used to prevent the recurrence of estrogen receptor positive (ER+) breast cancer in the adjuvant treatment of pre- and post-menopausal women, as well as for treatment of metastatic disease and ductal carcinoma in situ[1]. Tamoxifen is metabolized to several metabolites with greater anti-estrogenic potency including 4-hydroxy-tamoxifen and endoxifen[2]. Reaching therapeutic concentrations of these metabolites, particularly endoxifen, may be critical to the efficacy of tamoxifen treatment; patients above a therapeutic threshold have been reported to have lower recurrence rates than women with lower concentrations[3, 4].

The enzyme primarily responsible for metabolic conversion of tamoxifen to endoxifen is CYP2D6, but other enzymes and transporters are also active in this metabolic pathway[5]. Genetic variation in *CYP2D6* has a profound effect on endoxifen serum concentrations as it is the main pathway for the conversion of the primary tamoxifen metabolite, N-desmethyltamoxifen to endoxifen, and substantially contributes to the formation of 4-OH tamoxifen from tamoxifen[3][6]. Patients with genotypes that confer *CYP2D6* poor metabolizer (PM) phenotype have lower endoxifen concentrations than those with intermediate (IM), normal (NM, formerly referred to as extensive [EM]) or ultra-rapid (UM) metabolizer status[7].

Aside from CYP2D6, there are several enzymes and transporters known to play a role in the metabolism and/or distribution of tamoxifen and endoxifen. Prior studies have reported associations between tamoxifen and/or its metabolites for common functionally consequential single nucleotide polymorphisms (SNPs) in *CYP2C9*[4, 5, 8]and *CYP2C19*[4, 8, 9]. In addition to genetics, there are some patient specific factors such as height, weight, and the calendar date that may contribute to steady state endoxifen concentrations[10]. It may be possible to use genetic and clinical information to predict a patient's endoxifen concentration prior to initiation, and use this information to guide personalized tamoxifen dose adjustment to achieve therapeutic endoxifen concentrations. Several groups, including ours, have piloted this approach by dose escalating patients with low-activity *CYP2D6* genotypes and have made progress towards normalizing endoxifen concentrations without increasing treatment related toxicity[2, 11–14]. In addition, one study dose escalated patients solely based on baseline endoxifen[15]; however, these studies have been unable to achieve comparable endoxifen levels in *CYP2D6* PM patients suggesting other genetic data and clinical variables need to be incorporated into a prediction algorithm.

Further research is needed to determine the impact of SNPs in enzymes and transporters on endoxifen concentrations in order to improve the accuracy of personalized tamoxifen dosing algorithms. Prior pharmacogenetic studies have used a candidate SNP approach with limited allelic coverage. Our objective was to use a comprehensive genotyping approach that includes the vast majority of functionally consequential SNPs in the genes relevant to endoxifen pharmacokinetics (*CYP2C9*, *CYP2C19*, *CYP3A4*, *CYP3A5*)[4, 5, 8, 9, 16–20] to discover genetic predictors of steady-state endoxifen concentration in patients receiving tamoxifen treatment. We also included additional enzymes and transporters that have insufficient available information to rule out their involvement in endoxifen concentrations, several of which have been previously associated with tamoxifen efficacy and/or toxicity[21–23].

Methods

Patient Cohort

Patients included in this secondary analysis were originally enrolled in a prospective clinical study of *CYP2D6* genotype-guided tamoxifen dose escalation (UNC Lineberger Comprehensive Cancer Center 0801)[2]. Detailed inclusion and exclusion criteria were previously reported, but briefly: women 18 years of age and older and taking tamoxifen 20 mg for at least 4 months prior to study entry were eligible to enroll unless they had Eastern Cooperative Oncology Group (ECOG) performance status >2, impaired liver, bone marrow or kidney function, history of thromboembolic disease, prior liver or bone marrow transplant, received blood transfusions within 3 months of study entry, or were taking strong CYP2D6 inhibitors. Patients taking medications known to inhibit CYP2D6 including amiodarone, bupropion, cimetidine, haloperidol, indinavir, ritonavir, terbinafine, quinidine, duloxetine, paroxetine, or fluoxetine were excluded. Those taking weak inhibitors of CYP2D6 (venlafaxine, citalopram, sertraline, or escitalopram) were included. After an initial pilot study of 120 patients[2] the study was expanded to a total of 500 participants[24].

Sample and Data Collection

Patient demographic and treatment information including age, weight, menopausal status, self-reported race, duration of tamoxifen therapy, and concomitant medication were collected at study entry. Genomic DNA was isolated from the blood samples collected at entry into the clinical trial for germline genotyping. Baseline tamoxifen and metabolite concentrations were measured at baseline when all patients were taking tamoxifen 20 mg daily and had reached steady state. The following metabolites were measured in addition to tamoxifen: 4-OH-tamoxifen, N-desmethyl tamoxifen, and endoxifen. Concentrations were determined by liquid chromatography-mass spectrometry by collaborators at Indiana University using a previously described assay[2].

Genotyping and Activity Phenotype Prediction

At study enrollment, *CYP2D6* status was determined for each participant using the Roche AmpliChip™ CYP450 Test (Indianapolis, IN). Genotype was categorized into diplotypes (i.e. NM/IM, NM/PM, IM/IM, IM/PM) and then phenotypes (UM, NM, IM, PM) as previously described[25]. Phenotypes UM and NM were combined due to small number of

UM phenotypes and prior work in the cohort that confirmed differences in activity are negligible[25]. Residual DNA samples were genotyped for 36 additional genes, primarily CYP450 enzymes and drug transporters, on the iPLEX® ADME PGx Pro Panel by Agena Bioscience (San Diego, CA) for this secondary pharmacogenetic analysis. All genetic information underwent appropriate quality control. SNPs with genotyping call rate <95% and samples with a call rate of <90% across all genotyped SNPs were excluded from analysis. Of the 36 genes genotyped on the panel, the following 19 genes were chosen to be included in the analysis based on their potential contribution to tamoxifen metabolism or transport: *CYP2C9*, *CYP3A4*, *CYP3A5*, *ABCB1*, *SLOCO1B1*, *SULT1A1*, *SULT1A2*, *UGT2B7*, *UGT2B15*, *UGT2B17*, *CYP1A1*, *CYP1A2*, *CYP2A6*, *CYP2B6*, *CYP2C19*, *CYP2C8*, *CYP2E1*, *ABCC2* and *ABCG2*. Each allele was assigned a predicted activity determination (low, normal, high) based on published data and each patient's activity diplotype was translated into predicted activity phenotypes (poor, intermediate, normal or ultra-rapid) for analysis, as described in Supplemental Digital Content, Tables 1 and 2.

Statistical Analysis

Patients who were successfully genotyped and had baseline endoxifen concentration data measured on the modified assay used for the expansion cohort were included in the analysis. Steady state endoxifen concentrations underwent square root transformation to improve distributional normality. Predicted activity for each enzyme or transporter was analyzed assuming an additive effect with the number of levels (2, 3 or 4) corresponding to the number of phenotypes described in Supplemental Digital Content, Table 2. Clinical variables analyzed included age and weight as continuous variables, season of sample collection (January–March, April–June, July–September, October–December), CYP2D6 inhibitor status (none or weak CYP2D6 inhibitor), menopausal status (pre- or post-menopausal), and self-reported ethnicity (white, African-American, or other) as categorical variables. Associations between predicted activity phenotype or clinical variables and baseline steady state concentration for tamoxifen and its metabolites were analyzed using univariate linear regression. Following univariate analysis, genetic associations were adjusted for clinical covariates that were significant in the univariate setting. Multivariable models were constructed with each combination of CYP2C8, CYP2C9, and CYP2C19 activity phenotypes to assess the independent contribution of each gene. The most parsimonious model was selected based on Akaike Information Criterion (AIC), and R-squared values were estimated from the linear regression models. As a hypothesis generating secondary screen, the association of each predicted activity phenotype with tamoxifen, its other metabolites (4-OH-tamoxifen and N-desmethyl-tamoxifen) and their metabolic ratios was evaluated. An uncorrected p-value threshold of $p<0.05$ was considered statistically significant for all analyses, which were conducted in SAS v9.4 statistical software (Cary, NC).

Results

Patient and Demographic Data

After excluding subjects that were missing endoxifen concentration or genetic data, 302 patients were included in the final analysis (Figure 1). Patient demographic data including

age, weight, race, date of sample collection, *CYP2D6* diplotype, and concomitant use of a CYP2D6 inhibitor (i.e. citalopram, escitalopram, sertraline or venlafaxine) can be found in Table 1. The average age in this cohort was 52.8, 85% of the patients self-reported as Caucasian, and 45% were pre-menopausal. The *CYP2D6* phenotype distribution was similar to previously reported in this cohort with 37% having NM phenotype (NM/NM diplotype), 4% having PM phenotype (PM/PM diplotype) and the remaining ~60% being IM phenotype that can be subdivided into several diplotypes as reported in Table 1[25].

Univariate Associations of Clinical and Genetic Data

In the univariate analysis, an increase in steady-state endoxifen concentration was detected for patients with increased *CYP2C9* phenotype activity (Linear Regression β coefficient=0.20, 95% Confidence Interval: 0.01–0.39, p=0.04) and *CYP2C8* phenotype activity (β=0.22, 95% CI: 0.03–0.40, p=0.02, Table 2, Figure 2). None of the other genes analyzed were associated with endoxifen concentration in the univariate analysis, though there was a suggestive trend for *SLCO1B1* (p=0.07, Table 2) and *CYP2C19* (p=0.11, Table 2, Figure 2).

A significant association was found with higher weight associated with lower endoxifen concentration (β=−0.014, 95% CI: −0.020 − −0.008, p<0.0001, Figure 3). We also found that patients whose samples were collected in the fall (β=−0.55, 95% CI: −0.84 − −0.26, p=0.0002), summer (β=−0.55, 95% CI: −0.88 − −0.23, p=0.0009) or spring (β=−0.39, 95% CI: −0.71 − −0.07, p=0.02) had significantly lower endoxifen concentration compared with samples collected in the winter (Figure 3). No evidence of an association was found with other clinical variables: age, race, menopausal status, or concomitant administration of weak CYP2D6 inhibitors (Table 3).

Adjusted Associations for Genetic Variables

After adjustment for clinical variables with significant univariate associations (*CYP2D6* diplotype, weight and season) the association for CYP2C9 maintained significance (p=0.02) while the association for CYP2C8 was no longer significant (p=0.11). Interestingly, the association for CYP2C19 was nearly significant after adjustment (p=0.07) whereas the trend for SLCO1B1 disappeared (p=0.18, Table 2).

The trends seen with CYP2C8, CYP2C9, and CYP2C19 are likely due to known linkage disequilibrium between the high activity *CYP2C19*17* and the wild-type *CYP2C8* and *CYP2C9* (D'>0.9)[26]. Therefore, we constructed multivariable models including different combinations of the three genes adjusted for CYP2D6, weight, and season (Supplemental Digital Content, Table 3). Comparing the goodness of fit statistics (AIC), the optimal model included only CYP2C9 (p=0.002, AIC 728.2378, Supplemental Digital Content, Table 3). CYP2C9 maintained significance when included in a model with CYP2C19 (p=0.045) and was borderline significant in a model with CYP2C19 and CYP2C8 (p=0.07), but neither of these other genes maintained significance when included in a model with CYP2C9 (all p>0.05), suggesting that CYP2C9 drives the identified associations for all three genes. In the final multivariable model, the addition of CYP2D6 diplotype explained 15.3% of the

variability in steady-state endoxifen concentration while CYP2C9 (1.3%), weight (5.3%), and season (3.8%) explained relatively little of the residual variability (Table 4).

Secondary Screen of Genetic and Clinical Predictors of Tamoxifen, Metabolites, and Metabolite Ratios

Screening of other metabolites and metabolite ratios for hypothesis generation led to identification of associations with weight and season and two nominal associations with genetic factors. Similar to the association seen with endoxifen, greater weight was associated with lower concentrations of OH-tamoxifen (β=−0.0038, 95% CI: −0.0062 – −0.0015, p=0.0021, Supplemental Digital Content, Table 4) and N-desmethyltamoxifen (β=−0.025, 95% CI: −0.044 – −0.0048, p=0.017). In addition, OH-tamoxifen concentrations were lower in the fall (β=−0.21, 95% CI: −0.32 – −0.96, p=0.0004, Supplemental Digital Content, Table 4), and summer (β=−0.13, 95% CI: −0.26 – −0.0038, p=0.049) and N-desmethyltamoxifen concentrations were lower in the fall (β=−1.053, 95% CI: −1.99 – −0.12, p=0.031,) as compared to winter, similar to the associations detected for endoxifen. Screening predicted activity phenotype, higher SULT1A1 activity was nominally associated with greater endoxifen/4-OH-tamoxifen ratio (β =0.54, 95% CI: 0.04–1.04, p=0.03) and increased CYP2C8 activity was associated with a higher ratio of endoxifen to N-desmethyltamoxifen (β=0.01, 95% CI: 0.00, 0.02, p=0.04, Supplemental Digital Content, Table 5).

Discussion

The effect of genetic variability in CYPs and transporters, aside from *CYP2D6*, and clinical variables on steady-state endoxifen concentrations has not been thoroughly explored. CYP2D6 is thought to account for around 50% of the metabolism of tamoxifen to endoxifen[7], leaving a large portion of the metabolism unexplained. In this study, dozens of functionally relevant SNPs were genotyped to predict patient's phenotypic activity of nineteen CYPs and transporters, in order to determine whether this activity was associated with variability in endoxifen concentrations. Our findings further support previous analyses suggesting that genetic variation in CYP2C9, weight, and the season of sample collection contribute to this residual variability. Although there were intriguing trends for CYP2C8 and CYP2C19, multivariable models suggest these may be spurious associations caused by linkage disequilibrium with CYP2C9.

Our findings support previous work indicating a contribution of CYP2C9 to endoxifen production. Human liver microsomes homozygous for low-activity CYP2C9 genotypes have lower rates of 4-OH-tamoxifen formation than wild-type microsomes[18]. This *in vitro* finding has been confirmed in clinical cohorts of tamoxifen treated patients. Lower steady-state endoxifen[5] or 4-OH-tamoxifen[4] concentrations have been detected in patients carrying common, low-activity CYP2C9 polymorphisms (CYP2C9*2 and *3). Recently, Powers et al. utilized a more comprehensive CYP2C9 activity phenotyping approach by integrating uncommon low-activity variants (i.e. *4 and *11) and concomitant inhibitor treatment (i.e. fluoxetine and omeprazole), confirming that patients with diminished CYP2C9 activity have lower endoxifen concentrations[17]. Our results are in line with these previous findings, though we did not have information on CYP2C9 inhibitor co-

administration. Other studies that did not detect associations between CYP2C9 phenotype and concentrations of endoxifen or upstream metabolites were likely underpowered due to the rarity of low-activity CYP2C9 variants in patients of Asian descent[7, 8]. Other clinical analyses have reported associations between common functionally consequential polymorphisms in CYP2C19 (i.e. low-activity *2 and *3 and high-activity *17) and concentrations of tamoxifen and its metabolites[8, 9]. Our multivariable model suggests that CYP2C19 itself has a negligible, if any, contribution to endoxifen concentration and these associations can likely be attributable to linkage disequilibrium between the high activity *CYP2C19*17* and the wild-type *CYP2C8* and *CYP2C9*[26].

This study, similar to other reports examining BMI, found that higher body weight was associated with lower endoxifen[3, 4]. Body weight was utilized instead of body mass index because height information was unavailable. We also detected a significant association with season of sample collection, in which samples collected during the winter had higher concentrations of endoxifen than those collected in other seasons. Slightly higher concentrations of tamoxifen metabolites, but not tamoxifen itself, were also identified in the winter, suggesting this may be a metabolic phenomenon. Previously, two groups have reported the opposite effect, with samples collected in the spring and summer having greater endoxifen concentration than those collected in the winter[10, 27]. The mechanism for this seasonal effect is unclear as the original hypothesis, that seasonal sun-associated vitamin D induced CYP3A4 activity, was not confirmed by analyses of vitamin D and CYP3A activity in these publications[27]. The reason for the discrepancy in the direction of effect between our study and these prior publications is also not clear, but may have to do with geographical differences in sun exposure, as our study was conducted in North Carolina, USA. Also differences in the prevalence of vitamin D supplementation between cohorts could contribute, however we did not collect this information. One final possible explanation is increased tamoxifen adherence during winter months due to increased tolerability of hot flashes. Contrary to that hypothesis, tamoxifen concentrations were not different between the seasons. Lastly, we did not detect an association with CYP2D6 inhibitor co-administration, which is known to be a major determinant of endoxifen concentration[10, 28]. This is unsurprising, as patients taking strong or moderate CYP2D6 inhibitors were excluded from this study and relatively few patients (n=23) were taking weak inhibitors, which likely have a marginal effect on enzyme activity.

Several limitations of this analysis should be considered. The primary limitation is the lack of confirmation from prospective clinical trials that endoxifen serum concentration is associated with tamoxifen treatment efficacy, or perhaps toxicity, in breast cancer patients[29, 30]. Additionally, despite our attempt to perform a comprehensive assessment of the known, functionally consequential polymorphisms within genes putatively related to endoxifen concentration, it is likely that our panel did not include some relevant genes and some variants within the genes analyzed, particularly less common variants that have not yet been discovered. The panel utilized was as comprehensive as possible considering current knowledge in the field. The predicted functionality for polymorphisms was extrapolated from known data, but could have been incorrectly categorized. Further research efforts are needed, likely in well-controlled *in vitro* experiments, to estimate metabolic activity of individual alleles, perhaps accounting for substrate-dependent effects.

Our analysis explained approximately a quarter of the variability in endoxifen concentration, which is lower than previously reported for *CYP2D6* genotype[7]. One possible explanation is that our samples were collected irrespective of the timing of the last dose. Additionally, endoxifen concentration was measured at baseline, assuming patients were adherent to tamoxifen treatment, as self-reported. Tamoxifen adherence is highest within the first year of therapy, around 77–88%, and decreases over time[31]; our patient cohort was taking tamoxifen on average 0.8 years prior to study entry, suggesting adherence was relatively high. Lastly, we did not perform multiple comparisons correction, increasing the possibility of false positive findings; however, the agreement of our findings with prior literature somewhat alleviates this concern.

Conclusion

In this retrospective secondary analysis of data collected from a prospectively enrolled cohort, genetic variation that confers diminished CYP2C9 activity was associated with decreased steady-state endoxifen concentrations. In adjusted analyses, CYP2C9 activity seemed to be independently contributory while CYP2C8 and CYP2C19 were not associated with endoxifen concentration, likely due to known linkage disequilibrium. These results suggest that individualized treatment approaches should consider several genetic and clinical factors in addition to *CYP2D6* status to improve treatment efficacy. Further research is needed to integrate these genetic variables into an endoxifen prediction algorithm to guide personalized tamoxifen dosing. A combined analysis of existing datasets could be conducted to develop a more comprehensive and accurate algorithm. However, for these algorithms to be clinically useful endoxifen concentration needs to be definitively associated with tamoxifen treatment outcomes, ideally therapeutic efficacy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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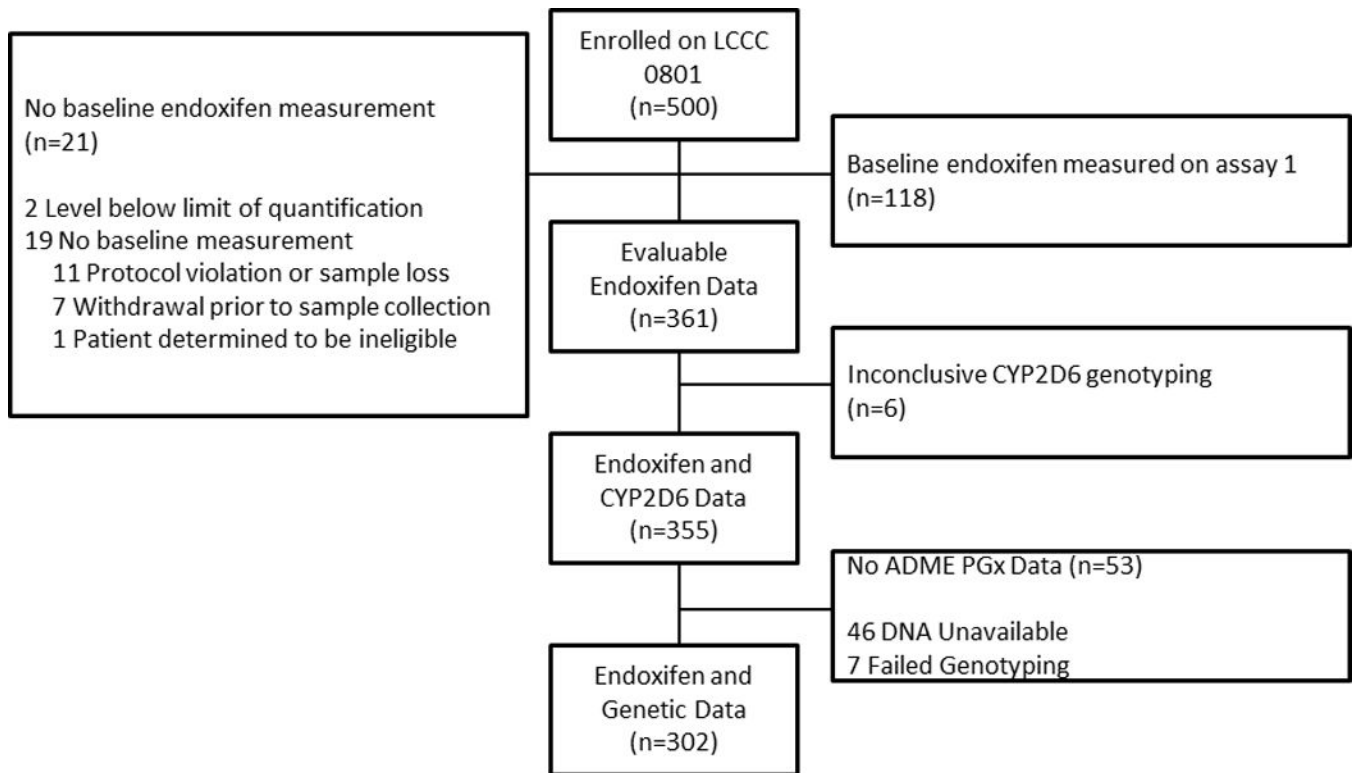
Figure 1. CONSORT Diagram of Patient Matriculation from Clinical Trial to this Pharmacogenetic Analysis

Of the 500 patients enrolled in the original clinical trial, 361 had evaluable endoxifen data, 355 had usable CYP2D6 genetic information, and 302 were successfully genotyped on the ADME PGx Pro Panel and were included in this analysis.
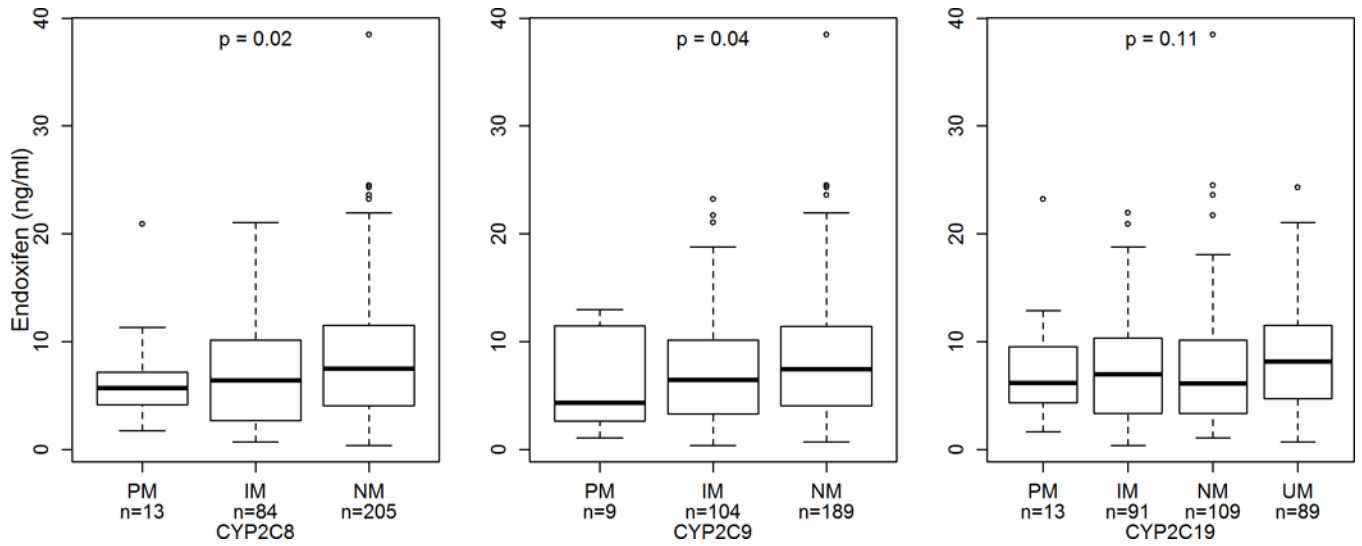
Figure 2. Endoxifen Concentration by CYP2C8, CYP2C9, *and* CYP2C19 Activity
Box and whisker plot (circles represent outliers) of steady-state endoxifen concentration stratified by predicted activity phenotype for *CYP2C8* (left), *CYP2C9* (middle), and *CYP2C19* (right). Endoxifen concentration increased as predicted phenotypic activity increased for each gene (p=0.02, p=0.04, p=0.11, respectively).
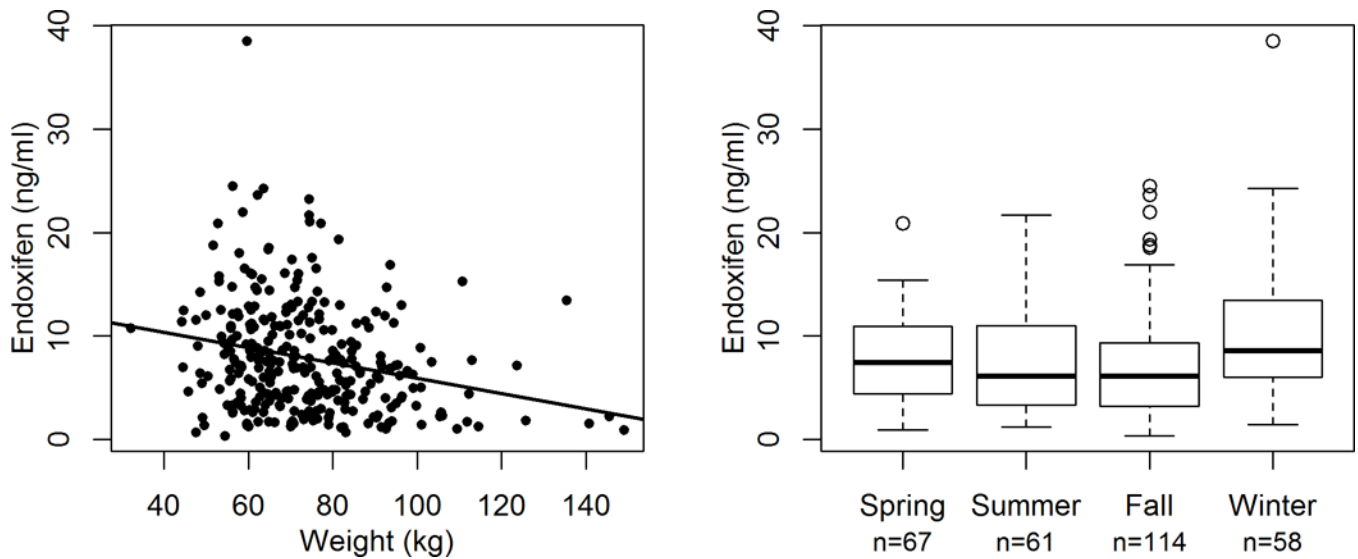
Figure 3. Association of Endoxifen Concentration with Clinical Variables
Steady-state endoxifen concentration was associated with body weight and the season of
sample collection. Figure 3 (left): Increased patient body weight (kg) was associated with
lower endoxifen concentrations ($\beta=-0.014$, p<0.0001). Figure 3 (right): Endoxifen
concentrations were lower in samples collected during fall ($\beta=-0.55$, p=0.0002) summer ($\beta=$
-0.55, p=0.0009) and spring ($\beta=-0.39$, p=0.02) compared with those collected during
winter.

Table 1

Patient Demographics

Clinical Variable	Level	Median (range) or n (%)
Age	Years	52.8 (24.8–95.1)
Weight	Kg	70.8 (32.1–149.1)
Self-Reported Race	Caucasian	253 (84.6%)
	African American	38 (12.7%)
	Other/Unknown	8 (2.7%)
Season of Baseline Sample Collection for Endoxifen Measurement	Winter (Jan–Mar)	58 (19.4%)
	Spring (April–June)	67 (22.3%)
	Summer (July–Sept)	61 (20.3%)
	Fall (Oct–Dec)	114 (38.0%)
Concomitant CYP2D6 Weak Inhibitor Use	Yes (total) citalopram escitalopram sertraline	23 (7.7%) 12 (4.0%) 10 (3.3%) 1 (0.33%)
	No	277 (92.3%)
Menopausal Status	Pre-menopausal	134 (44.7%)
	Post-menopausal	166 (55.3%)
Time on Tamoxifen Prior to Enrollment	Years	0.8 (0.3–9.7)
CYP2D6 Diplotype as defined in[26] (UM Alleles Considered NM alleles)	NM/NM	110 (37%)
	NM/IM	58 (19.5%)
	NM/PM	79 (26.6%)
	IM/IM	10 (3.4%)
	IM/PM	27 (9.1%)
	PM/PM	13 (4.4%)

Table 2

Mean Endoxifen Concentration by Metabolizer Status for All Genes Included in Analysis

	Mean Endoxifen (Std. Dev.) Stratified by Predicted Activity Phenotype in ng/mL				Univariate and Adjusted Association of Each Gene with Endoxifen Concentration	
Gene	Poor	Intermediate	Normal	Ultra-rapid	Unadjusted Association	Adjusted Association*
CYP1A1	3.19 (1.53) n=2	7.07 (4.66) n=52	8.05 (5.51) n=248		β = 0.20 (−0.06, 0.46) p=0.13	β = 0.18 (−0.05, 0.42) p=0.12
CYP1A2	9.57 (6.18) n=6	6.15 (3.76) n=22	8.13 (4.48) n=22	7.93 (5.53) n=252	β = 0.04 (−0.11, 0.20) p=0.61	β = −0.02 (−0.16, 0.12) p=0.80
CYP2A6	7.46 (9.71) n=5	7.65 (4.50) n=74	7.95 (5.57) n=220		B = 0.03 (−0.19, 0.25) p=0.79	β = 0.13 (−0.06, 0.33) p=0.18
CYP3A4	NA n=0	9.28 (4.99) n=14	7.78 (5.39) n=288		β = −0.33 (−0.83, 0.18) p=0.20	β = −0.35 (−0.80, 0.11) p=0.13
CYP3A5	7.71 (5.29) n=240	8.35 (5.40) n=54	8.51 (7.70) n=8		β = 0.06 (−0.16, 0.28) p=0.58	β = 0.05 (−0.14, 0.24) p=0.61
CYP2B6	8.20 (4.81) n=24	7.48 (5.53) n=127	8.11 (5.33) n=151		β = 0.06 (−0.11, 0.22) p=0.50	β = 0.01 (−0.14, 0.16) p=0.94
CYP2C8	6.71 (5.04) n=13	6.73 (4.29) n=84	8.38 (5.72) n=205		β = 0.22 (0.03, 0.40) **p=0.02**	β = 0.13 (−0.03, 0.30) p=0.11
CYP2C9	6.26 (4.82) n=9	7.18 (4.90) n=104	8.29 (5.61) n=189		β = 0.20 (0.01, 0.39) **p=0.04**	β = 0.20 (0.03, 0.37) **p=0.02**
CYP2C19	7.95 (5.66) n=13	7.25 (4.72) n=91	7.64 (5.97) n=109	8.71 (5.16) n=89	β = 0.10 (−0.02, 0.22) p=0.11	β = 0.10 (−0.01, 0.21) p=0.07
CYP2E1		8.27 (5.28) n=23	8.03 (5.40) n=84	7.72 (5.39) n=195	β = −0.05 (−0.22, 0.11) p=0.53	β = −0.10 (−0.26, 0.05) p=0.18
ABCB1	8.93 (7.53) n=37	7.90 (5.15) n=205	7.01 (4.41) n=60		β = −0.13 (−0.31, 0.06) p=0.19	β = −0.01 (−0.18, 0.16) p=0.92
ABCC2	9.30 (5.34) n=10	7.52 (5.42) n=69	8.29 (4.99) n=89	7.62 (5.61) n=134	β = −0.03 (−0.15, 0.09) p=0.63	β = −0.02 (−0.13, 0.08) p=0.65
ABCG2	12.82 (10.85) n=3	7.30 (4.28) n=50	7.90 (5.48) n=249		β = 0.01 (−0.25, 0.26) p=0.96	β = −0.08 (−0.31, 0.15) p=0.51
SLCO1B1	6.97 (4.21) n=62	7.76 (5.64) n=153	8.63 (5.57) n=87		β = 0.14 (−0.01, 0.29) p=0.07	β = 0.09 (−0.04, 0.22) p=0.18
SULT1A1	7.81 (6.59) n=45	8.35 (5.56) n=102	7.56 (4.51) n=111	7.45 (5.65) n=44	β = −0.04 (−0.16, 0.07) p=0.49	β = −0.00 (−0.11, 0.10) p=0.94
SULT1A2	7.72 (5.70) n=74	7.86 (5.31) n=144	7.94 (5.23) n=84		β = 0.01 (−0.14, 0.16) p=0.88	β = 0.06 (−0.07, 0.19) p=0.40
UGT2B7	8.38 (5.36) n=63	7.97 (5.49) n=166	7.11 (5.09) n=73		β = −0.12 (−0.28, 0.03) p=0.12	β = −0.11 (−0.25, 0.03) p=0.12

	Mean Endoxifen (Std. Dev.) Stratified by Predicted Activity Phenotype in ng/mL				Univariate and Adjusted Association of Each Gene with Endoxifen Concentration	
Gene	Poor	Intermediate	Normal	Ultra-rapid	Unadjusted Association	Adjusted Association*
					p=0.12	
UGT2B15	7.75 (4.48) n=75	7.88 (5.61) n=149	8.07 (5.82) n=71		β =−0.00 (−0.15, 0.15) p=0.98	β = 0.04 (−0.09, 0.18) p=0.52
UGT2B17	6.60 (5.48) n=35	8.27 (5.45) n=138	7.83 (5.25) n=122		β = 0.08 (−0.08, 0.24) p=0.30	β = 0.06 (−0.08, 0.20) p=0.41

*
P-values were adjusted for season, weight, and *CYP2D6* diplotype

Gray boxes denote phenotype groups that did not exist for that gene (See Supplementary Table 2)

Table 3

Univariate Association of Clinical Variables with Endoxifen Concentration

Clinical Variable		B Coefficient (95% Confidence Interval)	P-value
Weight (kg)		−0.014 (−0.020, −0.008)	**< 0.0001**
Age (10 years)		0.05 (−0.05,0.14)	0.31
Self-reported Race (vs. white)	African American	−0.13 (−0.45,0.18)	0.41
	Other	0.15 (−0.50,0.81)	0.65
Season of Sample Collection (vs. winter)	Fall	−0.55 (−0.84, −0.26)	**0.0002**
	Summer	−0.55 (−0.88, −0.23)	**0.0009**
	Spring	−0.39 (−0.71, −0.07)	**0.02**
Post-Menopausal (vs. pre)		0.14 (−0.08,0.35)	0.21
Concomitant CYP2D6 Weak Inhibitor Use		0.33 (−0.07,0.73)	0.11

Table 4

Final multivariable model of genetic and clinical variables associated with steady-state endoxifen concentration.

Clinical Variable		B Coefficient (95% Confidence Interval)	P-value	r² Contribution to Final Model
CYP2C9 Activity Phenotype		.20 (0.03, 0.37)	.025	1.3
Weight (kg)		−.013 (−.02, −.01)	<0.0001	5.3
Season of Sample Collection (vs. winter)	Fall	−.48 (−.74, −.22)	0.0004	3.8
	Summer	−.49 (−.79, −.19)	0.0017	
	Spring	−.41 (−.7, −.19)	0.0064	
CYP2D6 Diplotype as defined in [25] (vs. NM/NM, UM Alleles Considered NM alleles)	NM/IM	−.35 (−.61, −.08)	.0155	15.3
	NM/PM	−.49 (−.73, −.26)	<0.0001	
	IM/IM	−.87 (−1.4, −.34)	0.0015	
	IM/PM	−.96 (−1.3, −.61)	<0.0001	
	PM/PM	−1.32 (−1.79, −.85)	<0.0001	