

CYP2D6 Genotype, Antidepressant Use, and Tamoxifen Metabolism During Adjuvant Breast Cancer Treatment

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Background: The efficacy of tamoxifen therapy for the treatment of breast cancer varies widely among individuals. Plasma concentrations of the active tamoxifen metabolite endoxifen are associated with the cytochrome P450 (CYP) 2D6 genotype. We examined the effects of concomitant use of selective serotonin reuptake inhibitor antidepressants, which are CYP2D6 enzyme inhibitors commonly prescribed to treat hot flashes in women who take tamoxifen, and genotypes for genes that encode tamoxifen-metabolizing enzymes on plasma concentrations of tamoxifen and its metabolites. **Methods:** Eighty patients with newly diagnosed breast cancer who were beginning tamoxifen therapy (20 mg/day orally), 24 of whom were taking CYP2D6 inhibitors, were genotyped for common alleles of the CYP2D6, CYP2C9, CYP3A5, and sulfotransferase (SULT) 1A1 genes. Plasma concentrations of tamoxifen and its metabolites were measured after 1 and 4 months of tamoxifen therapy. Differences in plasma concentrations of tamoxifen and its metabolites between genotype groups were analyzed by the Wilcoxon rank sum test. All statistical tests were two-sided. **Results:** Among all women, plasma endoxifen concentrations after 4 months of tamoxifen therapy were statistically significantly lower in subjects with a CYP2D6 homozygous variant genotype (20.0 nM, 95% confidence interval [CI] = 11.1 to 28.9 nM) or a heterozygous genotype (43.1 nM, 95% CI = 33.3 to 52.9 nM) than in those with a homozygous wild-type genotype (78.0 nM, 95% CI = 65.9 to 90.1 nM) (both $P = .003$). Among subjects who carried a homozygous wild-type genotype, the mean plasma endoxifen concentration for those who were using CYP2D6 inhibitors was 58% lower than that for those who were not (38.6 nM versus 91.4 nM, difference = -52.8 nM, 95% CI = -86.1 to -19.5 nM, $P = .0025$). The plasma endoxifen concentration was slightly reduced in women taking venlafaxine, a weak inhibitor of CYP2D6, whereas the plasma endoxifen concentration was reduced substantially in subjects who took paroxetine (a potent inhibitor of CYP2D6). Genetic variations of CYP2C9, CYP3A5, or SULT1A1 had no statistically significant associations with plasma concentrations of tamoxifen or its metabolites. **Conclusion:** Interactions between CYP2D6 polymorphisms and coadministered antidepressants and other drugs that are CYP2D6 inhibitors may be associated with altered tamoxifen activity. [J Natl Cancer Inst 2005;97:30-9]

The selective estrogen receptor modulator, tamoxifen, has been widely used for more than 25 years for the endocrine treatment of all stages of hormone receptor-positive breast cancer (1). Tamoxifen is also approved by the United States Food and Drug Administration for the prevention of breast cancer in

women at high risk for developing the disease (2). The clinical effects of tamoxifen with respect to efficacy and toxicity vary widely among individuals. For example, among women with advanced breast cancer, roughly 35% of those with estrogen receptor-positive tumors do not respond to tamoxifen therapy, and all tumors that do respond eventually become resistant to tamoxifen treatment (1). Although tamoxifen therapy is associated with secondary benefits, such as improvement in lipid profiles and increases in bone mineral density in postmenopausal women, it is also associated with several adverse events, including rare venous thromboses and endometrial cancer and, more commonly, hot flashes (1). Because the risk of hot flashes is two- to threefold higher among women who take tamoxifen than it is for those who do not (3), selective serotonin reuptake inhibitor (SSRI) antidepressants are commonly prescribed to treat hot flashes in women who take tamoxifen. However, some SSRIs, such as paroxetine and fluoxetine, are known to inhibit cytochrome P450 (CYP) 2D6 (4), an enzyme that is important for the metabolism of many drugs, including tamoxifen.

The mechanisms of variable response to tamoxifen have been the subject of much scrutiny but remain obscure. Early attempts to link a clinical response to tamoxifen therapy with plasma tamoxifen concentrations revealed no statistically significant differences in outcomes between women who received 20 mg of tamoxifen daily and those who received 40 mg of tamoxifen daily, even though women in the 40-mg tamoxifen group had higher plasma tamoxifen concentrations than those in the 20-mg tamoxifen group (5). These results have been widely cited as evidence that plasma tamoxifen concentration is not a predictor of clinical outcome (6). However, it is now known that the overall pharmacologic action of tamoxifen in vivo is probably due, in part, to its conversion to active metabolites. Because there is strong evidence that tamoxifen is converted to antiestrogenic metabolites that are more potent than tamoxifen itself, one hypothesis is that altered patterns of metabolism of tamoxifen might contribute to interindividual variability in effects.

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Jordan et al. (7) demonstrated that hepatic metabolism of tamoxifen resulted in a statistically significant increase in its efficacy *in vivo*; they also showed for the first time that 4-hydroxytamoxifen, one of the human tamoxifen metabolites, is approximately 100 times more potent than tamoxifen as an estrogen antagonist *in vitro*. The tamoxifen metabolite N-desmethyltamoxifen is as potent as tamoxifen *in vitro* (8) but is more abundant in plasma of patients receiving tamoxifen treatment than tamoxifen itself (9). We have recently characterized the activity of another tamoxifen metabolite, 4-hydroxy-N-desmethyltamoxifen (endoxifen), which is present at notably higher concentrations than 4-hydroxytamoxifen in the plasma of breast cancer patients receiving chronic tamoxifen therapy (10). In a series of *in vitro* studies, we have shown that endoxifen exhibits the same potency and efficacy as 4-hydroxytamoxifen in suppressing estrogen-dependent breast cancer cell growth and gene expression (9).

Tamoxifen is metabolized extensively by human liver enzymes to several primary and secondary metabolites. Results of *in vitro* studies have implicated multiple CYP isoforms (e.g., CYP3A, CYP2D6, CYP2C9, CYP2C19, CYP2B6, and CYP1A2) in the biotransformation of tamoxifen to its primary metabolites (11). In addition, the limited data on the metabolism of primary metabolites to secondary metabolites suggest that sulfation is important for the excretion of hydroxylated tamoxifen metabolites from the liver (12). A comprehensive kinetic characterization of tamoxifen sequential metabolism *in vitro* demonstrated that CYP3A is the major CYP isoform responsible for the formation of N-desmethyltamoxifen, whereas the generation of 4-hydroxytamoxifen and endoxifen appeared to be predominantly catalyzed by CYP2D6 (Fig. 1) (13). Other CYP isoforms appear to play less important roles in tamoxifen metabolism *in vitro* (13).

In a pilot clinical trial involving 12 breast cancer patients who were taking adjuvant tamoxifen, we observed that the

plasma concentrations of endoxifen appeared to be influenced by the patient's CYP2D6 genotype (9). The CYP2D6 gene is a polymorphic gene with 46 reported allelic variants, many of which result in the loss of CYP2D6 enzyme function. The plasma concentrations of endoxifen were statistically significantly lower in patients who were carriers of nonfunctional CYP2D6 allelic variants compared with those who had two functional wild-type alleles. Furthermore, plasma endoxifen concentrations were lower in patients who were also taking paroxetine (9), an SSRI commonly prescribed for depression and, more recently, for the nonhormonal treatment of hot flashes, than in patients who were not taking paroxetine (3). These preliminary data suggested that plasma concentrations of tamoxifen metabolites might be directly affected by germline gene polymorphisms as well as by SSRIs. Although enzymes other than CYP2D6, including CYP3A, CYP2C9, and sulfotransferase (SULT) 1A1 have also been implicated in the metabolism of tamoxifen *in vitro* (11), the effects of allelic variants of the genes that encode them on metabolite formation *in vivo* remain unknown. To address these issues and to validate our early observations in a larger population of patients with less exclusive criteria, we initiated a prospective study to test the effects of commonly prescribed SSRIs and candidate gene genotypes on plasma concentrations of tamoxifen and its metabolites in hormone receptor-positive women who were taking tamoxifen as adjuvant treatment for newly diagnosed breast cancer.

SUBJECTS AND METHODS

Subjects

Eligible women were prospectively recruited from the Lombardi Comprehensive Cancer Center at Georgetown University Medical Center, from the Breast Oncology Program at the University of Michigan Comprehensive Cancer Center, and from the Indiana University Cancer Center. Pre- and postmenopausal women (≥ 18 years old) with newly diagnosed breast cancer who were starting tamoxifen as standard adjuvant therapy were included in this study. Patients were excluded if they had started tamoxifen therapy concurrently with adjuvant chemotherapy and/or adjuvant radiation therapy or if they were taking other adjuvant endocrine therapy. Other reasons for exclusion included current chronic corticosteroid therapy (previous use during adjuvant chemotherapy was permitted) and use of clonidine, bellergal, or megestrol acetate for hot flash therapy. Patients who were pregnant or lactating were also excluded from the study. Study participants were allowed to take vitamin E, SSRIs, or herbal remedies, provided that the participant had been taking the agent for at least 4 weeks and intended to continue taking the agent for at least the first month while on the study. The study protocol was approved by the institutional review boards of all three study sites. All subjects provided written informed consent before study entry.

Study Design

This study was carried out as part of an ongoing multicenter, open-label prospective observational trial that was designed to test associations between polymorphisms of candidate genes and tamoxifen clinical response, including adverse effects and secondary benefits. We present here the data that relate to genetic

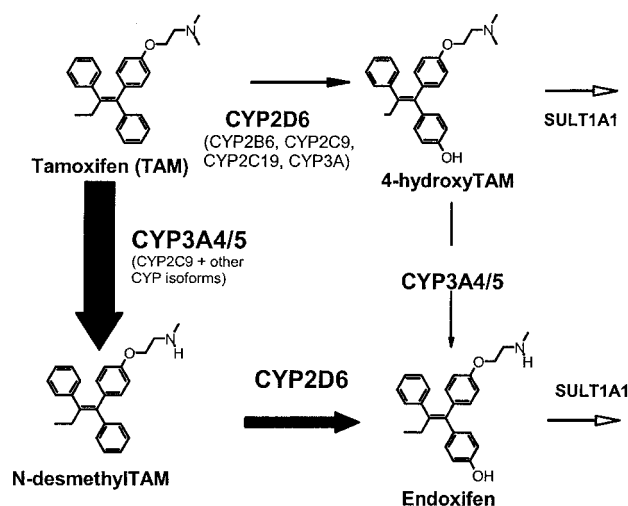


Fig. 1. Biotransformation of tamoxifen and its metabolites. Cytochrome P450 (CYP)3A4/5 is the major CYP isoform responsible for the formation of N-desmethyltamoxifen, whereas the generation of 4-hydroxytamoxifen and endoxifen are predominantly catalyzed by CYP2D6 (13). Other CYP isoforms, including CYP2C19, CYP2C9, and CYP2B6, appear to play less important roles in tamoxifen metabolism *in vitro* at therapeutically relevant concentrations (17). SULT1A1 has been proposed to bring about sulfation of 4-hydroxytamoxifen and may also play a role in endoxifen clearance.

polymorphisms in metabolic enzymes that contributed to tamoxifen metabolism and plasma concentrations of tamoxifen and its metabolites in this trial. Subjects were enrolled after they had completed all primary surgery, radiation, and adjuvant chemotherapy. Pretreatment medical histories, including a comprehensive list of current medications and the results of physical examinations and clinical laboratory examinations were obtained for each subject. At baseline (i.e., before tamoxifen therapy was initiated), each subject provided a blood sample (~10 mL), which was collected in a heparinized Vacutainer tube (Becton-Dickinson, Franklin Lakes, NJ), from which we extracted genomic DNA for genotyping analysis and isolated plasma. Tamoxifen (20 mg/day orally) had been prescribed for all subjects as part of adjuvant therapy and were followed up on an outpatient basis at 1, 4, 8, and 12 months after the start of tamoxifen therapy. During each follow-up visit, the subject's medical history and current medications were recorded, blood samples (5 mL) were collected in heparinized tubes, and plasma was separated within 1 hour of blood collection by centrifugation at 2060g. All samples (plasma and whole blood) were transferred to cryogenic vials (Corning, Cambridge, MA) and shipped to the Laboratory of the Division of Clinical Pharmacology at Indiana University on dry ice and were stored at -80°C pending analysis.

Measurement of Plasma Concentrations of Tamoxifen and Its Metabolites

We used a modification (9) of the method described by Fried and Wainer (14) to separate and quantify tamoxifen and its metabolites in plasma. Briefly, plasma samples (0.5 mL) were placed in 13-mL screw-cap glass tubes, and an internal standard (50 μ L of 10 μ g/mL propranolol in ethanol) was added to each tube and mixed by vortex. The mixture was made alkaline by adding 1 mL of 1 M NaOH-glycine buffer (pH 11.3) and extracted with 6 mL of hexane (95%)-isopropyl alcohol (5%) with shaking for 30 minutes. After centrifugation for 15 minutes at 2800g, the organic phase was removed and evaporated to dryness, and the residue was reconstituted with 100 μ L of the mobile phase (35% acetonitrile in 20 mM potassium phosphate buffer, pH 3). An aliquot of the reconstituted residue was injected into a high-performance liquid chromatography (HPLC) system.

Tamoxifen and its metabolites in plasma were separated by HPLC as follows. A semipermeable surface cyano guard column (1.0 \times 0.46 cm inside diameter [i.d.]; 5 μ m, 100 \AA particle size (Regis Chemical, Morton Grove, IL) was washed for 45 seconds with deionized water at a flow rate of 1 mL/minute and then with 35% acetonitrile in 20 mM potassium phosphate buffer (pH 3) at a flow rate of 1 mL/minute. We used a switching valve to redirect the eluent through a Rexchrom cyano analytical C₁₈ column (250 \times 4.6 cm i.d.; 5 μ m, 100 \AA particle size; Regis Chemical) equipped with a Rexchrom C₁₈ guard column (10 \times 3 mm; Regis Chemical) to a Beam Boost postcolumn photoreactor (ICT, Frankfurt, Germany) supplied with a 5-m reaction coil and a 254-nm UV lamp (Advanced Separation Technologies, Whippany, NJ), wherein the photoreaction converted tamoxifen and its metabolites to highly fluorescent phenanthrene derivatives (9). Gradient elution was used to separate the metabolites: the HPLC run was started with a mobile phase that consisted of 35% acetonitrile in 20 mM potassium phosphate

buffer (pH 3) at a flow rate of 1 mL/minute, and 20 minutes after the start of the HPLC run, the acetonitrile percentage was increased linearly over 15 minutes to 45% and held at that percentage for 10 minutes. The initial mobile-phase conditions were then resumed for 5 minutes. The operating temperature was ambient, and the flow rate was 1 mL/minute. The column eluent was monitored using fluorescent detection at an excitation wavelength of 256 nm and emission wavelength of 380 nm. HPLC instruments were controlled by CLASS-VP SP1 Chromatographic Software (version 7.1.1; Shimadzu Scientific Instruments, Columbia, MD) and included a solvent delivery module (model SCL-10A VP), an autoinjector (model SIL-10AD VP), a spectrofluorometric detector (model RF-10A XL), and a system controller (model SCL-10A VP) (all from Shimadzu Corporation, Analytical Instrument Division, Kyoto, Japan). We reduced the length of the water and mobile-phase washes of the guard column from 4 minutes (10) to less than 2 minutes. This modification, together with use of gradient HPLC elution, substantially improved the sensitivity of the assay by increasing the yield of the metabolites measured and provided an excellent separation of the metabolites.

We quantified plasma concentrations of tamoxifen and its metabolites by using the ratio of area under the curve (AUC) of tamoxifen or its metabolite to AUC of the internal standard and calibration curves that were constructed by spiking blank plasma (i.e., plasma obtained from subjects before they began taking tamoxifen) with known amounts of tamoxifen or its metabolites. For endoxifen and 4-hydroxytamoxifen, the limit of quantification was 0.1 ng/mL and the limit of detection was 0.05 ng/mL. For tamoxifen and N-desmethyltamoxifen, the limit of quantification was 1 ng/mL and the limit of detection was 0.5 ng/mL.

Genotyping Analysis

We used a QIAamp DNA blood Mini Kit (Qiagen, Valencia, CA) to extract genomic DNA from the leukocyte portion of whole blood and used the DNA to genotype variant alleles of four candidate genes whose protein products have been implicated in tamoxifen metabolism: CYP3A5, CYP2D6, CYP2C9, and SULT1A1. We screened for CYP2D6*1 (functional), and *3, *4, *5, and *6 (variant) alleles by using endonuclease-specific mutation analysis of a 4.7-kilobase pair DNA fragment that contained all nine exons of the CYP2D6 gene. That DNA fragment was amplified from the genomic DNA by using an expanded long-template polymerase chain reaction (PCR) and then used as a template to determine specific genetic variants by restriction fragment length polymorphism (RFLP) analysis as previously described (15,16). In addition, the presence of the CYP2D6*3 allele and confirmation of the CYP2D6*4, *5, and *6 alleles were determined by using a Taqman Allelic Discrimination Assay (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. CYP3A5*1 (functional) and *3 (variant) alleles were determined by allele-specific PCR using the method of Hiratsuka et al. (17,18) with slight modifications (19) and adaptation to a real-time SYBR green assay with an iCycler thermal cycler (BioRad Laboratories, Hercules, CA). We genotyped the CYP2C9*1 (functional) and the CYP2C9*2 and *3 (variant) alleles by using an RFLP-PCR assay as described by Nasu et al. (20). The digested PCR products of CYP2D6 and CYP2C9 gene variants were analyzed on a 2100 Bioanalyzer (Agilent Technologies, Rock-

ville, MD). We used an RFLP-PCR-based assay to genotype *SULT1A1**1 (functional) and *2 (variant) alleles, as described elsewhere (21), with the following minor modifications. The forward primer, I6F403 (5'-GTTGGCTCTG-CAGGGTCTCTAGGA-3'), and the reverse primer, I7R29 (5'-CCCAAACCCCGTACTGGCCAGACCC-3'), which are located in introns 6 and 7, respectively, were similar to those used by Coughtrie et al. (21) to detect the *SULT1A1**2 allele. *SULT* gene family members (*SULT1A1*, *SULT1A2*, and *SULT1A3*) exhibit high sequence homology. Thus, we designed this oligonucleotide primer pair to specifically amplify *SULT1A1* DNA at a high annealing temperature during the PCR reaction. PCR products were resolved by electrophoresis on 2% agarose gels (Invitrogen, Carlsbad, CA). We included DNA samples with known allele designations of *CYP2D6*, *CYP2C9*, *CYP3A5*, and *SULT1A1* on each gel.

Statistical Analysis

We used two-tailed paired Wilcoxon rank sum tests to examine the statistical significance of changes in the plasma concentration of tamoxifen and its metabolites from 1 to 4 months after tamoxifen therapy was initiated. We used simple linear regression tests to examine the statistical significance of associations among plasma concentrations of tamoxifen and its metabolites. Differences in the continuous demographic variables and in plasma concentrations of tamoxifen and its metabolites between groups with different genotypes were analyzed with two-tailed Wilcoxon rank sum tests. The effects of gene dosage on the plasma concentrations of tamoxifen and its metabolites were evaluated with a Jonckheere-Terpstra test (22). The statistical significance of associations between genetic polymorphisms and demographic and clinical categorical variables (i.e., race/ethnicity, history of chemotherapy, and menopausal status) were analyzed using chi-square tests. We used a bootstrap resampling algorithm (23) to adjust the *P* values because of the possibility of type I error inflation due to multiple testing. This procedure was implemented separately to compare changes in plasma levels of tamoxifen and its metabolite from 1 to 4 months after tamoxifen therapy was initiated and to test the effects of genotype on the plasma concentrations of tamoxifen and its metabolites 4 months after the initiation of tamoxifen therapy. This bootstrap algorithm allows assumptions of non-normality and unequal variance of the data. In addition, it utilizes the correlation information among predictors (genetic variables) and outcome variables (i.e., plasma concentrations of tamoxifen and its metabolites). All *P* values were two-sided and adjusted for multiple comparisons, and *P* values <.05 were considered statistically significant. All data are presented as mean values with 95% confidence intervals (CIs). The 95% confidence intervals were not adjusted for multiple testing to avoid masking of the true variance in concentrations of tamoxifen and its metabolites. All statistical tests were two-sided.

Sample Size and Power Calculations

To examine the statistical significance of differences in plasma concentrations of tamoxifen metabolite among groups with different genotypes, we used the approximate variability (i.e., 50% of the coefficient of variance) observed in the 4-month metabolite concentrations in our power calculations. We assumed that the allelic frequencies in our cohort were represen-

tative of those in the general population. Differences in plasma concentrations between genotype groups that were greater than 50% were considered to be clinically significant. In analyses that involved three genotype groups (i.e., *CYP2D6* [wild type/wild type, wild type/variant, variant/variant] and *SULT1A1* [*1/*1, *1/*2, *2/*2]), we defined a statistically significant gene dose effect as one that was associated with a 50% decrease or increase in the plasma concentrations of tamoxifen or its metabolites with every additional variant allele. Thus, we calculated that 80 subjects would provide at least 93% power to detect a statistically significant gene dose effect. In analyses that involved only two genotype groups (i.e., *CYP3A5* and *CYP2C9*, variant versus wild-type), we calculated that 80 subjects would provide 82% power to detect a 50% increase or decrease in the plasma concentrations of tamoxifen or its metabolites between the two genotype groups.

RESULTS

Demographic Characteristics of Patients

There were no statistically significant differences in demographic characteristics between different *CYP2D6* genotype groups (Table 1) or between subjects with different *CYP3A5*, *CYP2C9*, or *SULT1A1* alleles (data not shown). Before initiating tamoxifen therapy, 14 (17.5%) subjects were taking prescribed SSRIs; 4 months after initiating tamoxifen therapy, 23 (29%) subjects were taking prescribed SSRIs.

Frequencies of Variant alleles of *CYP2D6*, *CYP2C9*, *CYP3A5*, and *SULT1A1*

Because the majority (~93%) of the subjects enrolled in this study were white, we analyzed the alleles that are common in this population: *CYP2D6**1, *3, *4, *5, and *6; *CYP2C9**1, *2, and *3; *CYP3A5**1 and *3; and *SULT1A1**1 and *2. For each subject, we obtained conclusive genotype data for all the candidate alleles except for the *CYP2D6**5 allele. We could not obtain genotype data for the *CYP2D6**5 allele for 16 subjects because inadequate amounts of DNA were available for testing. Therefore, the allele frequency for *CYP2D6**5 was calculated from 64 subjects. The other allele frequencies were calculated from all 80 subjects. The frequencies of the *CYP2D6**1, *3, *4, *5, and *6 alleles were 77.8%, 1.3%, 18%, 2.3%, and 0.63%, respectively. These frequencies were similar to *CYP2D6* allele frequencies reported for other populations (24). The allele frequencies for *CYP3A5**1 and *CYP3A5**3 were 12% and 88%, respectively; the allele frequencies for *CYP2C9**1, *CYP2C9**2, and *CYP2C9**3 were 82.5%, 10.6%, and 6.9%, respectively. The allele frequencies for *SULT1A1**1 and *SULT1A1**2 were 68.7% and 31.3%, respectively. All *CYP2D6**4, *CYP2C9**2, *CYP2C9**3, *CYP3A5**3, and *SULT1A1**2 genotyping frequencies were in Hardy-Weinberg equilibrium (the sample size for the other variants was too small to reliably estimate Hardy-Weinberg equilibrium).

Plasma Concentrations of Tamoxifen and Its Metabolites in Women Taking Tamoxifen

Figure 2, A, shows the mean plasma concentrations of tamoxifen and its metabolites at 1 and 4 months after subjects initiated tamoxifen (20 mg/day by oral administration). There

Table 1. Patient characteristics*

Characteristic	Entire cohort (N = 80)	CYP2D6 genotype		
		Wt/Wt (n = 48)	Wt/Vt (n = 29)	Vt/Vt (n = 3)†
Age, y‡	57 (35–77)	54.5 (35–77)	55 (37–75)	56 (41–67)
Weight, kg‡	75.5 (50–127)	70.1 (53–127)	74 (56–108)	76 (60–99)
BMI, kg/m ² ‡	28.8 (18–48)	26.6 (18–48)	27 (22–40)	26 (21–36)
Race, No. (%)				
White	73 (91.2)	42 (87.5)	28 (96.6)	3 (100)
Black	4 (5.0)	3 (6.2)	1 (3.4)	0 (0)
Asian	2 (2.5)	2 (4.2)	0 (0)	0 (0)
Hispanic	1 (1.2)	1 (2.1)	0 (0)	0 (0)
Menopausal status, No. (%)				
Postmenopausal	47 (58.8)	31 (64.6)	15 (51.7)	1 (33)
Perimenopausal	7 (8.8)	4 (8.3)	2 (6.9)	1 (33)
Premenopausal	26 (32.5)	13 (27.1)	12 (41.4)	1 (33)
Previous chemotherapy, No. (%)				
Yes	39 (48.8)	24 (50)	13 (45)	2 (67)
No	41 (51.2)	24 (50)	16 (55)	1 (33)

*CYP = cytochrome P450; BMI = body mass index; Wt = wild type allele (CYP2D6 1); Vt = variant allele (CYP2D6*3, *4, *5, and *6 alleles).

†All three subjects carried two *4 alleles.

‡Values represent median (range).

was no statistically significant difference between the mean plasma concentration of tamoxifen after 1 month and after 4 months of therapy (377.4 nM versus 362.5 nM; difference = 14.9 nM, 95% CI = –13.9 to 43.7 nM, $P = .48$). However, mean plasma concentrations of the three tamoxifen metabolites we measured were statistically significantly higher after 4 months of tamoxifen therapy than after 1 month (N-desmethyltamoxifen: 654.9 nM versus 562.2 nM, difference = 92.7 nM, 95% CI = 13.0 to 172.4 nM, $P = .03$; endoxifen: 63.2 nM versus 53.9 nM, difference = 9.3 nM, 95% CI = 2.1 to 16.5 nM, $P = .016$; and 4-hydroxytamoxifen: 9.0 nM versus 8.2 nM, difference = 0.8 nM, 95% CI = 0.06 to 1.54 nM, $P = .0497$; paired Wilcoxon rank sum test). We therefore used the plasma concentrations obtained after 4 months of tamoxifen therapy to examine the influence of genotypes of CYP2D6, CYP2C9, CYP3A5, and SULT1A1 on the steady-state plasma concentrations of tamoxifen and its metabolites.

There were notable differences between the plasma concentrations of the different metabolites we measured. For example, the mean plasma concentration of N-desmethyltamoxifen at 4 months was 1.6-fold (95% CI = 0.95-fold to 2.86-fold, $P = .08$), 10.8-fold (95% CI = 2.54-fold to 45.87-fold, $P = .003$), and

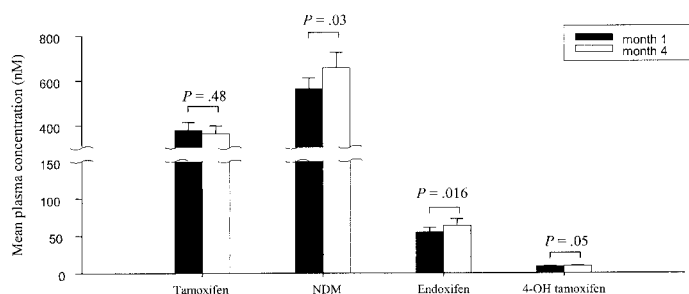


Fig. 2. Mean plasma concentrations of tamoxifen and its metabolites after 1 and 4 months of tamoxifen therapy. NDM = N-desmethyltamoxifen; 4-OH = 4-hydroxytamoxifen. **Error bars** correspond to 95% confidence intervals. Paired Wilcoxon rank sum tests were used to test the difference between mean plasma concentrations at 1 and 4 months after the initiation of tamoxifen therapy. P values were justified by bootstrapping samples 10 000 times among all four metabolites.

73.4-fold (95% CI = 8.1-fold to 668.0-fold, $P = .0004$) higher than those of tamoxifen, endoxifen, and 4-hydroxytamoxifen, respectively (Fig. 2). After 4 months of tamoxifen therapy, the mean plasma concentration of endoxifen was 6.8-fold (95% CI = 2.14-fold to 22.84-fold, $P = .002$) higher than that of 4-hydroxytamoxifen. The magnitude of this difference is similar to that which we previously reported (~11.7-fold) in a smaller pilot study (9). Mean plasma concentrations of both endoxifen and 4-hydroxytamoxifen were statistically significantly correlated with the mean plasma tamoxifen concentration ($R^2 = .19$ for endoxifen and 0.20 for 4-hydroxytamoxifen; $P < .001$); however, the mean plasma tamoxifen concentration explained only approximately 20% of the variability in mean plasma endoxifen and 4-hydroxytamoxifen concentrations.

Association Between CYP2C9, CYP3A5, and SULT1A1 Genotypes and Plasma Concentrations of Tamoxifen and Its Metabolites

We next examined associations between CYP2C9, CYP3A5, and SULT1 genotypes and the plasma concentrations of tamoxifen and its metabolites in women receiving tamoxifen therapy. For the analysis of CYP2C9 variants, we compared women who were homozygous for the wild-type alleles (Wt/Wt genotype group) with women who were either heterozygous (Wt/Vt genotype group) or homozygous for the variant alleles (Vt/Vt genotype group) because only two variant-allele homozygotes were identified. There was no statistically significant difference in the mean plasma concentrations of tamoxifen or any of its metabolites between these two CYP2C9 genotype groups (Table 2). This was the case even when we took into account the use of potent CYP2C9 inhibitors, such as amiodarone, by the subjects (data not shown).

For the analysis of the CYP3A5 variants, we compared subjects who had at least one functional CYP3A5 allele (i.e., those with the CYP3A5*1/*3 and the CYP3A5*1/*1 genotypes) with subjects who had no functional CYP3A5 allele (i.e., those with the CYP3A5*3/*3 genotype) because only two subjects carried the homozygous CYP3A5*1/*1 genotype. The mean plasma concentrations of endoxifen were

Table 2. Plasma concentrations of tamoxifen and its metabolites at 4 months of tamoxifen therapy in subjects with CYP2D6, CYP2C9, CYP3A5, and SULT1A1 genotype*

Genotype group	N	Mean concentration, nM (95% CI)			
		Endoxifen	4-Hydroxytamoxifen	N-desmethyltamoxifen	Tamoxifen
CYP2D6					
Wt/Wt	48	78.0 (65.9 to 90.1)	9.5 (8.4 to 10.6)	653.4 (562.5 to 744.3)	372.5 (321.2 to 423.8)
Wt/Vt†	29	43.1 (33.3 to 52.9)	8.3 (6.7 to 9.9)	687.3 (570.6 to 804.0)	353.3 (301.2 to 405.4)
Vt/Vt‡	3	20.0 (11.1 to 28.9)	7.1 (1.2 to 13.0)	664.1 (298.7 to 1029.5)	288.9 (172.9 to 404.9)
<i>P</i>		<.001	.86	.62	.92
CYP2C9					
Wt/Wt	55	63.4 (52.1 to 74.7)	8.9 (7.8 to 10.0)	648.3 (560.8 to 735.8)	349.8 (257.4 to 442.2)
Vt§	25	62.7 (47.6 to 77.8)	9.2 (7.4 to 11.0)	670.0 (555.7 to 784.3)	391.6 (335.2 to 448.0)
<i>P</i>		.87	.81	.90	.34
CYP3A5					
*1/**	17	82.0 (56.2 to 107.8)	9.7 (7.3 to 12.1)	655.3 (474.6 to 836.0)	402.3 (290.5 to 514.1)
*3/*3¶	63	58.1 (49.3 to 66.9)	8.7 (7.7 to 9.7)	654.8 (579.8 to 729.8)	352.4 (316.9 to 387.9)
<i>P</i>		.09	.57	.99	.98
SULT1A1					
*1*1	36	59.1 (46.4 to 71.8)	8.9 (7.4 to 10.4)	668.9 (572.2 to 765.6)	360.2 (307.1 to 413.3)
*1*2	38	65.1 (50.9 to 79.3)	8.8 (6.4 to 11.2)	667.6 (558.4 to 776.8)	377.0 (321.8 to 432.2)
*2*2	6	74.9 (47.2 to 102.6)	10.3 (5.7 to 14.9)	493.0 (37.1 to 948.9)	286.7 (177.6 to 395.8)
<i>P</i>		.73	.83	.58	.98

*The Jonckheere-Terpstra test was used to test the hypothesis that there are gene dosage effects of CYP2D6 and SULT1A1 on tamoxifen metabolite concentrations, and the Wilcoxon rank sum test was used to test the effects of CYP2C9 and CYP3A5. *P* values were justified by bootstrapping samples 10 000 times among all four metabolites and four genotypes. The 95% confidence intervals were not justified; hence the true tamoxifen metabolite sample variances are not concealed. CYP = cytochrome P450; SULT = sulfotransferase; CI = confidence interval; Wt = wild-type allele; Vt = variant allele.

†Wt/Vt group included subjects who carried at least one *3 allele (N = 2), *4 allele (N = 23), *5 allele (N = 3), or *6 allele (N = 1).

‡All were homozygotes of the CYP2D6 *4/*4 genotype.

§Includes the CYP2C9 *2 and *3 alleles.

||*1/** indicates that subjects in this group had at least one functional CYP 3A5 allele (i.e., CYP3A5 *1). This group includes 15 subjects with the CYP3A5 *1/*3 genotype and two subjects with the CYP3A5 *1/*1 genotype.

¶Indicates subjects who carry CYP3A5 *3/*3 genotype. They did not have any functional CYP3A5 alleles.

higher for subjects who carried at least one functional CYP3A5 allele than for those who carried no functional alleles, but the differences did not reach statistical significance (Table 2). The mean plasma tamoxifen concentration was statistically significantly higher for the five subjects who were taking calcium channel blockers that are known CYP3A inhibitors than it was for the remaining 73 subjects who had complete medication records and were not taking any CYP3A inhibitors (539.6 nM versus 344.7 nM, difference = 194.9 nM, 95% CI = 11.0 to 379.0 nM, *P* = .044). However, the use of CYP3A inhibitors was not associated with statistically significant alterations in the mean plasma concentrations of endoxifen, 4-hydroxytamoxifen, and N-desmethyltamoxifen among our cohort. In addition, there was no statistically significant association between the SULT1A1*2 genotype and the mean plasma concentrations of tamoxifen or its metabolites (Table 2). Additional analyses of gene–gene interactions between all candidate genotypes did not identify any subgroups of genotypes that had statistically significant associations with mean plasma concentrations of tamoxifen or its metabolites (data not shown).

Association Between CYP2D6 Genotypes and Plasma Concentrations of Tamoxifen and Its Metabolites

We tested for the presence of the CYP2D6*1, *3, *4, *5, and *6 alleles in all 80 subjects in this study and found that three subjects were homozygous for the *4 allele, 23 subjects were *1/*4 heterozygotes, three subjects were *1/*5 heterozygotes, two subjects were *1/*3 heterozygotes, and one

subject was a *1/*6 heterozygote. The remaining 48 subjects were homozygous for the wild-type allele (CYP2D6*1). The CYP2D6*4 allele results in a splicing defect, the CYP2D6*3 and *6 alleles lead to translation frameshifts, and the CYP2D6*5 allele causes deletion of the entire CYP2D6 gene; none of these four variants produces functional CYP2D6 enzyme (25). Thus, in our analysis of the effect of CYP2D6 genotypes on plasma concentrations of tamoxifen and its metabolites, we combined the subjects who were *1/*3, *1/*4, *1/*5, or *1/*6 heterozygotes together into a wild-type/variant group (Wt/Vt). Table 2 summarizes the mean plasma concentrations of tamoxifen and its metabolites after 4 months of tamoxifen therapy in subjects with different CYP2D6 genotype groups. CYP2D6 genotype was not statistically significant associated with mean plasma concentrations of tamoxifen, 4-hydroxytamoxifen, or N-desmethyltamoxifen. Subjects who were CYP2D6*1/*3, *1/*4, *1/*5, or *1/*6 heterozygotes (Wt/Vt genotype group) had mean plasma endoxifen concentrations that were 55% (95% CI = 16.9% to 147.4%) of those of subjects who were homozygous for the wild-type CYP2D6 genotype (Wt/Vt concentration versus Wt/Wt concentration: 43.1 nM versus 78.0 nM), and subjects who were CYP2D6*4/*4 homozygotes (Vt/Vt genotype group) had mean plasma endoxifen concentrations that were 26% (95% CI = 1.0% to 638.6%) of those of subjects who were homozygous for the wild-type CYP2D6 genotype (Vt/Vt versus Wt/Wt: 20.0 nM versus 78.0nM) (Table 2; Fig. 3, A) (*P* = .0002, *R*² = .23 for gene-dose effect).

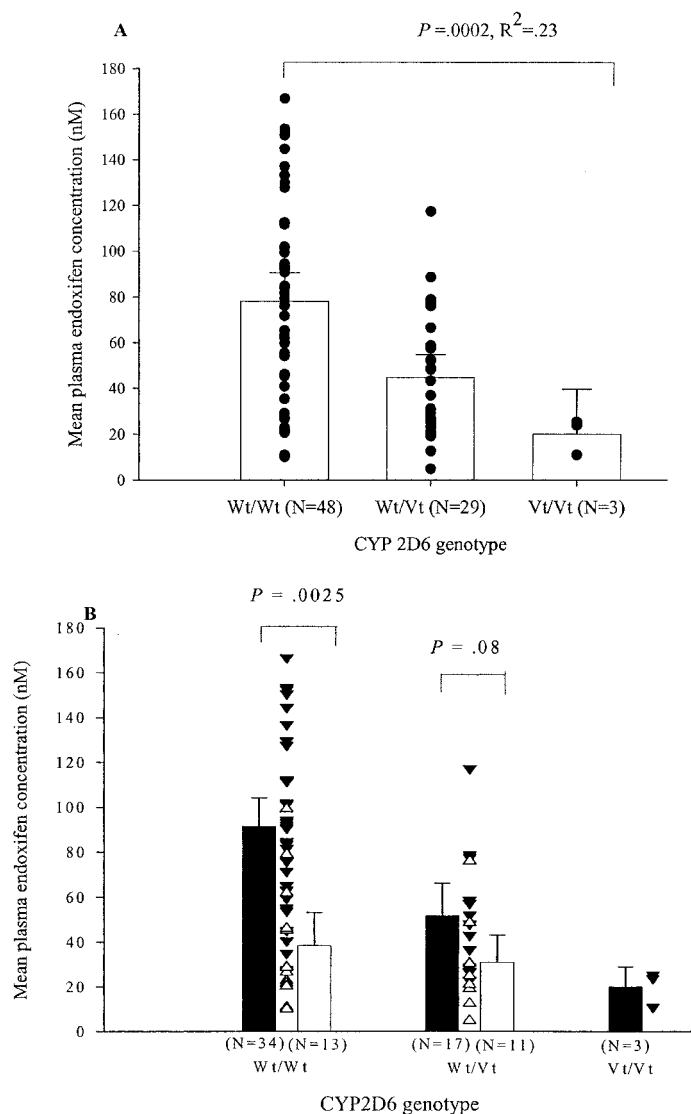


Fig. 3. Associations of cytochrome (CYP) 2D6 genotype and concomitant use of CYP2D6 inhibitors with plasma endoxifen concentration. **A)** Circles indicate individual plasma concentrations. Error bars correspond to 95% confidence intervals. Wt/Wt: absence of any CYP2D6 *3, *4, *5, or *6 variant alleles. Wt/Vt: subjects with one *3 (N = 2), *4 (N = 22), *5 (N = 3), or *6 (N = 1) variant allele. Vt/Vt: subjects carried two CYP2D6 *4 variant alleles. Using a two-tailed Jonckheere–Terpstra test we found a statistically significant gene-dose effect relationship (bootstrap adjusted and unadjusted $P = .0002$, and $P = .00003$, respectively). **B)** Associations of CYP2D6 genotype and inhibitors with mean plasma endoxifen concentration after 4 months of tamoxifen treatment. Solid bars and inverted filled triangles represent group means and individual concentrations, respectively, for subjects who were not receiving any CYP2D6 inhibitors; open bars and open triangles represent group means and individual concentrations, respectively, for subjects who were taking at least one CYP2D6 inhibitor. P values were bootstrap-justified and obtained by a two-tailed Wilcoxon rank sum test. Wt/Wt group = subjects who did not carry any *3, *4, *5, or *6 alleles; Wt/Vt group = subjects who carried one *3, *4, *5, or *6 allele; Vt/Vt group = subjects who were homozygotes for the *4/*4 genotype. Error bars indicate 95% confident intervals.

Effect of CYP2D6 Inhibitors on Plasma Concentrations of Endoxifen

The plasma endoxifen concentrations in patients who were homozygous for the wild-type allele were highly variable (Fig. 3, A). Although rare variants in the CYP2D6 gene might have

contributed to this large interindividual variability, it was also possible that the variability was due to exposure to drugs that inhibit the CYP2D6 enzyme. To test this latter possibility, we examined the association between the use of known CYP2D6 inhibitors and mean plasma endoxifen concentration. In our cohort of 80 breast cancer patients, 78 women had complete medication records 4 months after they had initiated tamoxifen therapy; of these, 24 women (30.8%) were taking medications considered to be CYP2D6 inhibitors based on results of published drug interaction studies (www.drug-interactions.com), including paroxetine, fluoxetine, sertraline, citalopram, amiodarone, and metoclopramide. Among subjects who carried the Wt/Wt genotype, the mean plasma endoxifen concentration for those who were using CYP2D6 inhibitors was 58% lower than that for those who were not using CYP2D6 inhibitors (38.6 nM versus 91.4 nM, difference = -52.8 nM, 95% CI = -86.1 to -19.5 nM, $P = .0025$) (Fig. 3, B). Among subjects who were heterozygous for a nonfunctional CYP2D6 allele (i.e., the Wt/Vt group), the mean plasma endoxifen concentration for those who were using CYP2D6 inhibitors was 38% lower than that for those who were not taking CYP2D6 inhibitors (31.0 nM versus 51.7 nM, difference = -20.7 nM, 95% CI = -42.7 to 1.31 nM, $P = .08$) (Fig. 3, B). Only three subjects were homozygotes for the CYP2D6*4/*4 genotype, and none of those three subjects was taking CYP2D6 inhibitors. Among the 54 subjects who were not taking a CYP2D6 inhibitor concomitantly with tamoxifen therapy, plasma concentrations of endoxifen were associated with the CYP2D6 genotype, as shown in Fig. 3, B ($P = .0001$). Among subjects who were taking CYP2D6 inhibitors, there was no statistically significant difference in the mean plasma concentration of endoxifen between subjects with the CYP2D6 Wt/Wt and Wt/Vt genotypes (38.6 nM versus 31.0 nM, difference = 7.6 nM, 95% CI = -6.2 to 21.4 nM, $P = .45$).

Associations Between the Use of Selective Serotonin Reuptake Inhibitors and Plasma Endoxifen Concentration

Among the 78 subjects for whom complete medication data were available, 23 (30%) were taking one of the following SSRIs: paroxetine (n = 10), sertraline (n = 4), citalopram (n = 4), fluoxetine (n = 2), and venlafaxine (n = 3). The inhibition constants for the inhibition of CYP2D6 by paroxetine (the most potent inhibitor), fluoxetine, sertraline, citalopram, and venlafaxine (the least potent inhibitor) are 0.05, 0.17, 1.5, 7, and 33 $\mu\text{mol/L}$, respectively (4,26). Figure 4 depicts the comparison between plasma endoxifen concentrations in subjects who were not taking any CYP2D6 inhibitors (and who were of either CYP2D6 Wt/Wt or CYP2D6 Vt/Vt genotype) with those who were taking venlafaxine, sertraline, or paroxetine. These data illustrate the fact that venlafaxine, a documented weak inhibitor of CYP2D6 (4), appeared to have very little effect on plasma endoxifen concentrations. By contrast, the mean plasma endoxifen concentration for subjects who took paroxetine was substantially lower than that of subjects with the CYP2D6 Wt/Wt genotype who were not taking a CYP2D6 inhibitor or of subjects who were taking venlafaxine. The mean plasma endoxifen concentration for subjects who took paroxetine was similar to that for subjects with the CYP2D6 Vt/Vt genotype who were not taking a CYP2D6 inhibitor. The mean plasma endoxifen concentration for subjects who were taking sertraline was intermediate between the two groups (i.e., those taking venlafaxine and

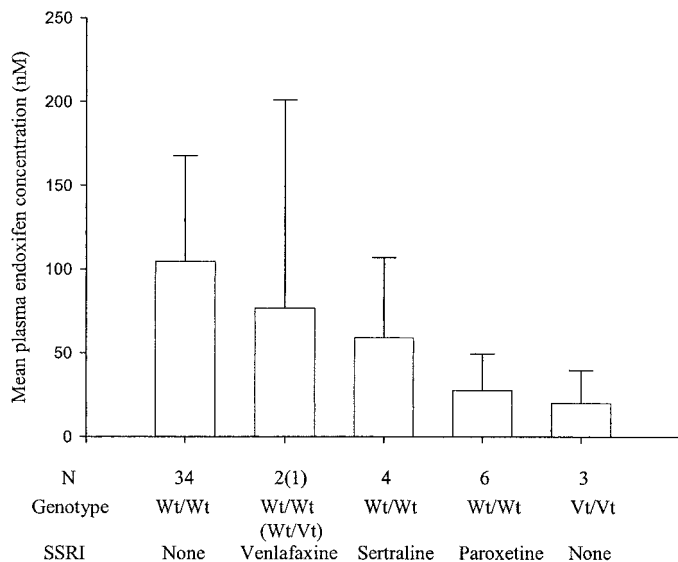


Fig. 4. Associations between commonly used antidepressants and plasma endoxifen concentration. Wt/Wt no inhibitors group included 34 subjects who did not carry any CYP2D6 *3, *4, *5, or *6 variant alleles and also did not take any CYP2D6 inhibitors. The venlafaxine group included three subjects who had the Wt/Wt genotype (N = 2) and Wt/Vt (N = 1). The sertraline group included four Wt/Wt subjects. The paroxetine group included six Wt/Wt subjects. Three subjects who carried the Wt/Vt genotype and were taking paroxetine were excluded from the analysis. The Vt/Vt group included three subjects who were homozygotes for the CYP2D6 *4/*4 genotype. Error bars correspond to 95% confidence intervals.

those taking paroxetine). The strength of the individual SSRI data on venlafaxine, paroxetine, and sertraline is in exact concordance with *in vitro* data, which affords biologic plausibility to the idea that the SSRIs act via CYP2D6 to lower the endoxifen concentration.

DISCUSSION

This study is the first large-scale clinical trial to examine the influence of multiple genetic polymorphisms and drug interactions on the plasma concentrations of tamoxifen and its active metabolites. We demonstrated a strong association between CYP2D6 activity and plasma endoxifen concentrations in women with breast cancer: the plasma concentrations of endoxifen were strongly associated with the CYP2D6 genotype, but not with the genotypes of the other candidate genes we tested, and with the use of concomitant medications that are known to inhibit the CYP2D6 enzyme.

In this prospective observational study, we also confirmed a prior report (27) indicating that steady-state plasma concentrations of tamoxifen metabolites are achieved after longer times than steady-state plasma concentrations of tamoxifen. The reason for this observation is unclear because no formal pharmacokinetic studies after administration of these metabolites to humans have been published. However, differences in tamoxifen metabolite elimination half-life, distribution volume, and formation rate may contribute to the difference between tamoxifen and its metabolites in the time required to reach steady-state plasma levels in response to chronic tamoxifen therapy. We also found that the mean steady-state plasma concentration of endoxifen was 6.8 times higher than that of 4-hydroxytamoxifen and that plasma endoxifen concentrations were associated with the

CYP2D6 genotype. Concomitant use of CYP2D6 inhibitors, including SSRIs, was associated with a reduction in the mean plasma concentration of endoxifen, and inhibitor potency was directly related to the mean plasma concentration of endoxifen. The magnitude of the reduction in plasma endoxifen concentration associated with CYP2D6 inhibitor use also depended on the CYP2D6 genotype: among women who carried Wt/Wt alleles, the use of CYP2D6 inhibitors was associated with an average 58% reduction in plasma endoxifen concentration, whereas among women who carried the Wt/Vt genotype CYP2D6 inhibitor use was associated with a mean 38% decrease. It is of note that none of the three subjects who were homozygous for the CYP2D6*4 allele was taking CYP2D6 inhibitors, an observation that raises the interesting possibility that these subjects could not tolerate CYP2D6 substrates, such as SSRI antidepressants. Our results confirm and extend the observations we made in a smaller pilot trial, which suggested that paroxetine interacts with the CYP2D6 genotype to alter tamoxifen metabolism (9). The differences we observed in plasma endoxifen concentrations between subjects who were and were not taking different SSRIs are important because a surprisingly high number of subjects (30%) were taking prescribed SSRI antidepressants; thus, patients taking some SSRIs (e.g., paroxetine) may have lower response to tamoxifen therapy that results from reduced formation of endoxifen.

Our results also highlight the role of CYP3A isoforms in tamoxifen metabolism *in vivo*. Subjects who were taking CYP3A inhibitors, such as calcium channel blockers, had higher steady-state mean plasma tamoxifen concentrations than subjects who were not taking CYP3A inhibitors, suggesting that CYP3A enzyme activity is important in the elimination of tamoxifen. Subjects who carried at least one CYP3A5*1 allele had higher plasma endoxifen concentrations than those who did not have any functional CYP3A5 allele (CYP3A5*3/*3 genotype), but that difference was not statistically significant.

Our finding that mean plasma concentrations of endoxifen were higher than mean plasma concentrations of 4-hydroxytamoxifen suggests that endoxifen may play a more important role than 4-hydroxytamoxifen in suppressing breast cancer growth *in vivo*. It also suggests that variations in plasma endoxifen concentrations that are associated with CYP2D6 gene polymorphisms and the coadministration of drugs that inhibit CYP2D6 may affect tamoxifen's antitumoral efficacy or its side effects. This hypothesis requires further testing in clinical trials in which it is possible to record the clinical outcome of treatment with tamoxifen in groups with different genotypes. We believe that this is an important hypothesis to test because genetic polymorphisms of CYP2D6 that abolish or impair CYP2D6 enzyme function are common in all ethnic groups, even though the allele frequencies differ among different ethnic groups. For example, the CYP2D6*2, *3, *4, *5, *6, *10, and *41 alleles are common in white subjects, the CYP2D6*2 and *17 alleles are common in black subjects, and the CYP2D6*10 allele is most common in Asian subjects (28). Among these common alleles, *3, *4, *5, and *6 result in an absence of functional CYP2D6 protein, whereas *2, *10, *17, and *41 result in CYP2D6 enzyme with reduced catalytic activity or altered substrate specificity (25). In this study, we focused on the *3, *4, *5, and *6 alleles because these four variant alleles account for approximately 97% of nonfunctional CYP2D6 variants in white populations (29). We plan to study the effect of rare null alleles or reduced-function

CYP2D6 alleles on tamoxifen metabolism in vivo in the context of larger sample size ($N = \sim 300$) from our ongoing multicenter trial. Given the high prevalence rate of CYP2D6 genetic polymorphisms in all ethnic groups and the widespread use of SSRIs and selective serotonin and norepinephrine reuptake inhibitors among breast cancer survivors for the treatment of depression and hot flashes (30–32), our findings may be relevant to a large number of women who take tamoxifen.

The importance of our findings hinges on the clinical relevance of changes in tamoxifen metabolite concentrations. In an effort to improve the generalizability of our data, we have studied a larger patient population than that studied in our pilot trial (9) with minimal interference with their routine clinical care and with very few exclusion criteria (Table 1). Women with a range of ages, weights, different histories of previous chemotherapy, different menopausal stages, and taking a wide variety of medications were included in this study. Our study does have certain limitations. First, we did not genotype all CYP2D6 alleles reported so far. Second, we obtained evidence of medication usage by patient reports. However, although this is an imperfect approach, it was the only practical means of obtaining this information available to us.

Our results offer insight into the differential effects of antidepressants on steady-state endoxifen concentrations and may also have implications for other drugs that interact with CYP2D6, of which there are many (www.drug-interactions.com). We found that more potent CYP2D6 inhibitors had greater effects on plasma endoxifen concentrations. Thus, knowledge of a drug's ability to inhibit CYP2D6 enzyme activity may help clinicians to anticipate clinically important drug interactions that could lower a patient's plasma endoxifen concentration. Our results also provide pharmacogenetic and pharmacokinetic bases for predicting tamoxifen metabolism and the formation of tamoxifen metabolites, including endoxifen, in vivo. Although other endocrine agents, such as the aromatase inhibitors, are of increasing importance in the treatment of breast cancer (33), it is likely that tamoxifen will remain an important agent for the foreseeable and perhaps distant future, especially in premenopausal women in whom aromatase inhibitors are unlikely to be useful, and in the prevention setting, which involves a large fraction of premenopausal women. Likewise, in light of recent data suggesting that estrogen therapy increases the risk of new breast cancers and may increase the risk of breast cancer recurrence in previously affected women (34), SSRIs are likely to gain increasing importance as therapeutic alternatives to estrogen for the treatment of hot flashes in patients with breast cancer as well as in unaffected women, in addition to their already established role in treatment of depression and other psychiatric illnesses (3,35). It is possible that testing of CYP2D6 genetic variants and careful attention to use of CYP2D6 inhibitors may help identify a group of women who may experience greater benefit from tamoxifen and/or who might benefit more from treatment with one SSRI over another. Firm clinical recommendations about which SSRI to use and whether genotype predicts clinical response of tamoxifen must await results from definitive clinical trials that include outcomes such as toxicity, secondary benefits, and, importantly, breast cancer recurrence and mortality.

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NOTES

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