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Evaluation of CYP2D6 and Efficacy of Tamoxifen and Raloxifene in Women Treated for Breast Cancer Chemoprevention: Results from the NSABP P-1 and P-2 Clinical Trials

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Abstract

Background—Controversy exists regarding the association between CYP2D6 enzyme activity and tamoxifen effectiveness in the adjuvant treatment of invasive breast cancer; however this association in the primary prevention of breast cancer is unknown.

Methods—We performed a nested case-control study in the context of the NSABP P-1 and P-2 prevention trials to determine the impact of *CYP2D6* genotype, CYP2D6 inhibitor use, as well as metabolizer status (*CYP2D6* genotype combined with CYP2D6 inhibitor use), on breast cancer events. Women who developed breast cancer (both non-invasive and invasive) while on five years of SERM therapy (cases) were matched to controls free of breast cancer. Comprehensive *CYP2D6* genotyping was performed for alleles associated with absent (*3, *4, *5, *6), reduced (*10, *17, *41), and increased (*1XN and *2XN) enzyme activity. Information regarding the use of CYP2D6 inhibitors was recorded.

Results—591 cases were matched to 1126 controls and DNA was genotyped in >97%. In patients treated with tamoxifen, there was no association of *CYP2D6* genotype [OR(extensive/poor metabolizer): 0.90; 95% CI 0.46-1.74, p=0.74), use of a potent CYP2D6 inhibitor (OR 0.92;

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95% CI 0.575-1.486), or CYP2D6 metabolizer status (OR 1.03; 95% CI 0.669-1.607) with breast cancer occurrence. Likewise, there was no association between any CYP2D6 metabolism parameter with breast cancer events in raloxifene treated patients.

Conclusions—In the NSABP P1 and P2 clinical trials, alterations in CYP2D6 metabolism are not associated with either tamoxifen or raloxifene efficacy.

Keywords

tamoxifen; breast cancer; prevention; CYP2D6; polymorphism

Background

There are two selective estrogen receptor modulators (SERM's) approved by the US FDA for the chemoprevention of breast cancer. These approvals were based on two double blind studies involving more than 33,000 women. The first was the placebo-controlled National Surgical Adjuvant Breast and Bowel Project (NSABP) P-1 trial, (1, 2) which demonstrated a nearly 50% reduction in the risk of developing breast cancer comparing 5 years of tamoxifen with placebo control. The second was the NSABP P-2 trial, (3, 4) which compared tamoxifen with raloxifene, and which demonstrated similar efficacy but a significantly lower risk of serious side-effects in favor of raloxifene.

Because not all women benefit from SERM therapy, and because of the increase in serious side effects (venous thromboembolic and uterine cancer), there is great interest in identifying biomarkers associated with drug effect (either efficacy or side-effects). One such focus is related to the pharmacogenetics of drug metabolism. Tamoxifen, a weak anti-estrogen, is extensively metabolized to potent anti-estrogens, 4-hydroxy tamoxifen and 4-hydroxy N-desmethyl tamoxifen (endoxifen), the latter of which is considered the most abundant and active tamoxifen metabolite (5, 6). While multiple Cytochrome P450 enzymes contribute to the formation of endoxifen, the rate limiting step is the CYP2D6-mediated oxidation of n-desmethyl tamoxifen (6, 7) and common genetic variations in *CYP2D6* and/or drug-induced inhibition of CYP2D6 enzyme activity are associated with significant reductions in endoxifen concentrations in tamoxifen treated humans (8).

In the adjuvant and metastatic treatment of invasive breast cancer, there have been numerous retrospective studies demonstrating both positive, (9) (10) (11–17) and negative associations (18–27) with regard to CYP2D6 metabolism and tamoxifen efficacy. While several small studies evaluated the association between CYP2D6 genotype and tamoxifen efficacy in the area of chemoprevention, (17, 28) these studies were limited by small sample sizes. We performed a nested case-control to analyze the association between *CYP2D6* genotype, CYP2D6 inhibitor use as well as the combination of both (CY2D6 metabolizer status) with breast cancer events in women who received tamoxifen or raloxifene in the P-1 and P-2 prevention trials.

Methods

Source of Patients

This research was performed after approval by local Institutional Review Boards in accordance with assurances filed with and approved by the Department of Health and Human Services (NCT00967239). Cases and controls were selected from the tamoxifen arm in the P-1 trial (NCT00066573) and from both the tamoxifen and raloxifene arms in the P-2 trial (NCT00003906). The P2 clinical trial enrolled only postmenopausal women (in contrast to P1 that enrolled pre and postmenopausal patients). Therefore, this biomarker study focused on subjects who were 50 years of age or older at time of entry. Cases were females

who experienced either invasive or non-invasive (ductal carcinoma in situ) breast cancer following the treatment with either tamoxifen or raloxifene. Controls were women who did not experience either of these events. A nested matched case-control design was used, with matching on the following factors: 1) trial and treatment arm (P-1 tamoxifen, P-2 tamoxifen, P-2 raloxifene); 2) age at trial entry (matched within 5 years), 3) 5-year predicted breast cancer risk based on the Gail model (<2.00%, 2.01-3.00, 3.01-5.00, >5.01), 4) history of lobular carcinoma in situ (yes vs. no); 5) history of atypical hyperplasia in breast (yes vs. no); 6) time on study (controls were required to be on study at least as long as the time to diagnosis of the breast event for the matched case). Because 94.2% of the participants on P-1 and P-2 treated with tamoxifen or raloxifene were Caucasian, our analysis was restricted to only Caucasians. Matching was done in a 2:1 fashion with a total of 591 cases matched to 1126 controls. The match analysis included 51 cases (each matched to one control) and 539 cases (each matched to two controls).

CYP2D6 Genotyping

The DNA samples were plated in triplicate into 384-well plates, with cases and controls randomly distributed across the plates. Patient DNA samples were genotyped for the *CYP2D6* alleles associated with null (*3, *4, *6) and reduced (*10, *17 and *41) CYP2D6 enzyme activity using the Applied Biosystems' Taqman SNP Genotyping Assays (Foster City, CA) according to the manufacturer's instructions. The *5 *CYP2D6* allele (associated with null enzyme activity) and the determination of multiple gene copies (associated with increased CYP2D6 enzyme activity) was assessed using the Applied Biosystems' Taqman Copy Number Assay. Briefly, 3 ng of DNA was dried in a plate well then a 5-uL reaction containing forward and reverse primers along with allele-specific probes was added. The polymerase chain reaction and fluorescence measurements were performed using the ABI Prism 7900HT Real Time system or the BioRad CFX384 Real Time PCR detection system. All analysis included negative and positive controls previously determined by validated PCR and sequencing techniques.

CYP2D6 Drug Inhibitor Use

The medical records of P1 and P2 participants were reviewed to determine if they were prescribed either a weak or potent CYP2D6 inhibitors as outlined in Supplementary Table 1. For each drug, documentation was obtained whether the patient was taking the drug at baseline (yes, no) and also if the drug was used during the course of follow-up (yes, no). If a CYP2D6 inhibitor was started during the course of follow-up, the start date was recorded. Because stop dates were not collected, determination of drug duration was not possible.

CYP2D6 metabolism definition

CYP2D6 genotype was assessed for the most common alleles associated with null, intermediate, extensive, and ultra-rapid enzyme function (Table 1). The predicted phenotype using genotype alone (for patients not receiving an inhibitor) is listed in Table 2, using a previous published classification system (10).

CYP2D6 Metabolizer Classification

We performed comprehensive CYP2D6 phenotyping (termed CYP2D6 metabolizer) using a combination of CYP2D6 genotype and documentation (yes/no) regarding use of a CYP2D6 inhibitor, based on prior studies demonstrating greater prediction of steady state endoxifen concentrations (29) when both CYP2D6 genotype and CYP2D6 inhibitor were used for prediction of steady state endoxifen concentrations. Table 2 illustrates how the genotype and drug inhibitor use were combined to create this metabolizer class. Subjects that were on multiple drugs at entry or during follow-up were classified according to the drug with the

greatest degree of CYP2D6 enzyme inhibition. Because only the starting date (and not stop date) of each drug was recorded, we assumed that CYP2D6 metabolizer classification (related to genotype) changed only if the following held: a subject was recorded as taking a potent CYP2D6 inhibitor at study entry, or those who started a potent CYP2D6 inhibitor within the first two years of follow-up. The CYP2D6 metabolizer status of patients receiving a weak CYP2D6 inhibitor (at baseline or starting within the first two years of SERM therapy) changed only as illustrated in Table 2. If a subject was not on any drug at entry or within the first two years, and the subject was classified as no inhibitor drug use for the classification used in Table 1.

For the statistical analyses of the association of CYP2D6 metabolizer class with case-control status, the ultra-rapid and extensive categories were combined. Conditional logistic regression modeling for the matched case-control design was used to examine whether the odds of disease was associated with *CYP2D6* genotype, inhibitor use, and metabolizer status for each drug. Assuming a Type-I error rate of 0.05 and the estimated standard error of our observed odds ratio for poor vs. extensive metabolizers., we had 80% power to detect an odds ratio of at least 1.54. Two-sided P-values < 0.05 were considered statistically significant. Analysis was performed using SAS (Version 9.1.3, SAS Institute Inc., Cary, NC). To aid interpretations of the effect size, 95% confidence intervals are provided.

Results

Characteristics of the Patients

93 percent of women enrolled in the P1 and P2 clinical trials provided a blood sample for the pharmacogenetic study including 89 percent of the cases and 95 percent of the controls. Table 3 demonstrates the number of observed genotypes and minor allele frequencies for each allele. Table 4 presents the characteristics of the cases and controls and show that they were well balanced.

Association of CYP2D6 genotype with breast cancer events in patients treated with either tamoxifen or raloxifene

We observed no association between CYP2D6 genotype and the development of a breast cancer event in either the tamoxifen or raloxifene arms (Table 5).

Association of CYP2D6 inhibitor use with breast cancer events in patients treated with either tamoxifen or raloxifene

In this study, a total of 196 women were recorded as either taking (at baseline) or starting a potent CYP2D6 inhibitor within the first 2 years (tamoxifen, n=95; raloxifene, n=101). Additionally, 119 patients were recorded as either taking at baseline or starting a weak CYP2D6 inhibitor within the first 2 years (tamoxifen, n=66; raloxifene, n=53). We observed no association between the odds of developing breast cancer and the use of either a potent inhibitor with tamoxifen or raloxifene or the use of a weak inhibitor with tamoxifen or raloxifene (Supplementary Table)

Association of CYP2D6 Metabolizer Status (based on genotype and drug inhibitor) with Breast Cancer Events in patients treated with either Tamoxifen or Raloxifene

Patients were grouped into poor, intermediate, and extensive CYP2D6 metabolizers using both CYP2D6 genotype and CYP2D6 inhibitor use (Table 2). In this analysis, both UM and EM phenotype categories were grouped together. No statistically significant associations were observed between CYP2D6 metabolizer status and development of breast cancer in patients treated with either tamoxifen or raloxifene (Table 6).

Discussion

In a comprehensive analysis of postmenopausal patients enrolled into the NSABP P-1 and P-2 clinical trials, we identified no statistically significant association of CYP2D6 genotype, inhibitor use, or metabolizer status with the odds of a breast cancer event, in patients treated with tamoxifen or raloxifene, either when both were combined or were considered individually.

Considering the adjuvant therapy setting in women who have developed breast cancer, there have been conflicting data regarding the association between CYP2D6 metabolism (either genotype and/or drug inhibitor use) and tamoxifen treatment outcome (30). This heterogeneity is well illustrated in the data recently presented from 3 large adjuvant clinical breast cancer trials, wherein no association of CYP2D6 genotype with breast cancer recurrence was observed in the ATAC and BIG 1-98 clinical trials (31, 32). In contrast, within the ABCSG 8 clinical trial, CYP2D6 poor metabolizers were identified to have a statistically significantly increase in the odds of breast cancer recurrence in patients who received tamoxifen monotherapy, but not those who received tamoxifen followed by anastrozole (33). Furthermore, a secondary analysis of tamoxifen pharmacokinetic data from the subgroup of women taking tamoxifen (n=1,370) within the dietary and lifestyle intervention study (WHEL), demonstrated a significantly increased risk for disease recurrence in tamoxifen treated patients with low steady state concentrations of endoxifen (<5.9 ng/ml), as compared to patients with concentrations >5.9 ng/ml (34). While >75% of CYP2D6 PM were represented in this lowest endoxifen concentration category, CYP2D6 genotype alone was not associated with an increased risk of recurrence in the WHEL study.

In the setting of tamoxifen chemoprevention, a previous report from a small subgroup of patients enrolled in the Italian prevention study demonstrated an association between CYP2D6 genotype and the development of breast cancer in women treated with tamoxifen (17). Furthermore, in a recent “case-only” analysis of patients enrolled only within the NSABP P1 clinical trial, Dunn et al evaluated 39 “candidate” genes and demonstrated that the CYP2D6 C1111T allele, individually and within a CYP2D6 haplotype was associated with treatment arm, suggesting a potential role of CYP2D6 in tamoxifen response. However, in this latter analysis, important CYP2D6 null and reduced function alleles were not included in this study, nor did the study account for the use of CYP2D6 inhibitors. Furthermore, Dunn et al evaluated patients enrolled only in the P1 trial, which enrolled both pre and postmenopausal patients. In contrast, this current analysis was restricted to postmenopausal patients, and the majority of cases (>85%) derived from the P2 clinical trial. Thus, the role of CYP2D6 metabolism in the prevention of premenopausal breast cancer is unknown.

Overall, while the data suggest that variability in the concentration of endoxifen may affect the breast cancer outcomes of patients who receive tamoxifen for the adjuvant treatment of invasive ER positive breast cancer, this does not seem to hold in women receiving tamoxifen as chemoprevention. Furthermore, given the null effects of CYP2D6 observed in this study, the role of variation in other Cytochrome P450 and conjugating enzymes is unlikely to have substantial effects of tamoxifen efficacy.

While endoxifen concentrations appear to substantially contribute to the inhibition breast cancer cell growth *in vitro*, (35) the concentrations of tamoxifen and its metabolites necessary for the *in vivo* prevention of breast cancer are unknown. The great heterogeneity with regard to both the positive and negative data in the adjuvant setting suggest that additional factors affect the ability of both normal and malignant breast tissue to respond to tamoxifen. Recent data suggest that expression of ERβ, a known tumor suppressor, may

affect the concentrations of endoxifen needed for growth inhibition (36). Notably, in vitro studies demonstrated that even low concentrations of endoxifen (observed in tamoxifen treated patients with deficient CYP2D6 activity) markedly inhibited estrogen-induced cell proliferation rates in the presence of ERβ. Given that the expression of ER beta is substantially higher in normal compared with malignant breast tissue (37), endoxifen concentrations may be less critical in the setting of chemoprevention than in the setting of invasive disease. Lastly, new data suggest that the most abundant tamoxifen metabolite, N-desmethyl tamoxifen, and endoxifen, can both inhibit the aromatase enzyme (38). While the clinical implications of these findings are still uncertain, the variability in tamoxifen drug response phenotypes are likely related to a complex relationship between tumor subtype and the relative concentrations of tamoxifen and its metabolites.

In summary, in high-risk postmenopausal women receiving either tamoxifen or raloxifene for the prevention of breast cancer, alterations in CYP2D6 metabolism are not associated with the development of breast cancer.

Translational Relevance

Although variation in CYP2D6 metabolism has been reported to be associated with tamoxifen clinical outcomes in the treatment of invasive breast cancer, there are few data with regard to the association between CYP2D6 metabolism and breast cancer prevention. A large nested case-control analysis of the NSABP P1 and P2 clinical trials demonstrated no association between *CYP2D6* genotype, CYP2D6 inhibitor use or the combined CYP2D6 metabolizer status. These data strongly suggest that variations in the active metabolites of tamoxifen are not related to the efficacy of tamoxifen in the prevention setting. Further research in this setting of SERM chemoprevention should focus on unbiased analysis of global genetic variation and its association with the development of breast cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

CYP2D6 alleles according to predicted enzyme function.

Enzyme Function	Allele
Poor (PM)	*3, *4, *5, *6
Intermediate (IM)	*10, *17, *41
Extensive (EM)	*1, *2
Ultrarapid (UM)	*1XN, *2XN

Table 2

CYP2D6 metabolizer status based on genotype and drug inhibitor use.

CYP2D6 metabolizer status	***CYP2D6* genotype**	**CYP2D6 drug inhibitors used**	
		Weak inhibitor	**Potent inhibitor**
Ultra-rapid	EM/UM, UM/UM	no	no
Extensive	EM/EM	no	no
	EM/UM, UM/UM	yes	no
Intermediate	EM/IM	no	no
	EM/EM	yes	no
	IM/IM	no	no
	EM/PM	no	no
	EM/IM	yes	no
	EM/PM	yes	no
	IM/IM	yes	no
	PM/IM	no	no
Poor	unknown	no	yes
	any genotype	no	yes
	PM/IM	yes	no
	PM/PM	no	no
	PM/PM	unknown	

Table 3

Number of observed genotypes and minor allele frequencies (q)

	Genotype counts	Minor allele frequency
*CYP2D6 *3*		0.02
Wt/Wt	1697	
Wt/*3	53	
*3/*3	0	
*CYP2D6 *4*		0.20
Wt/Wt	116	
Wt/*4	566	
*4/*4	76	
CYP2D6 copy number		0.03
>2	75	
2	1590	
1 (*5)	88	
0	2	
*CYP2D6 *6*		0.01
Wt/Wt	1688	
Wt/*6	42	
*6/*6	2	
*CYP2D6 *10*		0.02
Wt/Wt	1674	
Wt/*10	52	
*10/*10	2	
*CYP2D6 *17*		0.00
Wt/Wt	1683	
Wt/*17	4	
*17/*17	0	
*CYP2D6 *41*		0.10
Wt/Wt	1390	
Wt/*41	316	
*41/*41	20	

Table 4

Patient Characteristics

	Controls (N=1126)	Cases (N=591)
NSABP Trial		
P1	138 (12.3%)	79 (13.4%)
P2	988 (87.7%)	512 (86.6%)
Type of Breast Event		
Invasive Breast Cancer	0 (0%)	452 (76.5%)
DCIS	0 (0%)	139 (23.5%)
Treatment		
Tamoxifen	595 (52.8%)	318 (53.8%)
Raloxifene	531 (47.2%)	273 (46.2%)
Age (yrs) at entry		
Mean (SD)	59.9 (7.30)	59.9 (7.28)
Median	59.0	59.0
< 55	276 (24.5%)	146 (24.7%)
55–59	327 (29%)	170 (28.8%)
60–64	252 (22.4%)	136 (23%)
65+	271 (24.1%)	139 (23.5%)
5-year predicted breast cancer risk		
Mean (SD)	4.8 (2.40)	4.9 (2.50)
Median	4.2	4.5
<=2.00%	63 (5.6%)	33 (5.6%)
2.01–3.00%	236 (21%)	121 (20.5%)
3.01=5.00%	349 (31%)	183 (31%)
>5.00%	478 (42.5%)	254 (43%)
History of LCIS at entry		
NO	918 (81.5%)	479 (81%)
YES	208 (18.5%)	112 (19%)
History of Atypical Hyperplasia at entry		
NO	863 (76.6%)	435 (73.6%)
YES	263 (23.4%)	156 (26.4%)
Number of First-degree Relatives with Breast Cancer		
0	338 (30%)	198 (33.5%)
1	544 (48.3%)	268 (45.3%)
2+	244 (21.7%)	125 (21.2%)
Time (months) on Tamoxifen or Raloxifene		
Mean (SD)	47.2 (18.78)	29.5 (20.30)
Median	60.0	27.5

Table 5

Association of CYP2D6 Genotype with odds of Breast Cancer risk

Treatment	Status	EM/EM (baseline)	EM/IM	IM/IM	EM/PM	PM/IM	PM/PM	Missing Genotype data
Tamoxifen	Controls	243	86	8	195	29	32	2
	Cases	129	46	4	97	23	16	3
	Odds Ratio	1	0.994	1.096	0.947	1.461	0.895	
	95% CI	-	(0.651–1.518)	(0.309–3.889)	(0.677–1.325)	(0.787–2.711)	(0.462–1.736)	
	p-value	-	0.979	0.888	0.75	0.229	0.743	
Raloxifene	Controls	226	61	4	175	34	28	3
	Cases	107	47	4	72	22	21	0
	Odds Ratio	1	1.671	2.417	0.87	1.321	1.597	
	95% CI	-	(1.052–2.653)	(0.513–11.379)	(0.605–1.25)	(0.747–2.335)	(0.872–2.924)	
	p-value	-	0.03	0.264	0.451	0.338	0.129	
Total	Controls	469	147	12	370	63	60	5
	Cases	236	93	8	169	45	37	3
	Odds Ratio	1	1.257	1.566	0.916	1.386	1.213	
	95% CI	-	(0.922–1.713)	(0.601–4.085)	(0.717–1.171)	(0.914–2.103)	(0.778–1.891)	

Table 6

Association of CYP2D6 Metabolizer Status (based on genotype and drug inhibitor) with Breast Cancer Risk

Treatment	Status	Extensive (baseline)	Intermediate	Poor	Metabolizer status unknown
Tamoxifen	Controls	202	295	93	5
	Cases	111	156	48	3
	Odds Ratio	1	0.957	0.942	
	95% CI		(0.697–1.315)	(0.609–1.458)	
	p-value		0.787	0.789	
Raloxifene	Controls	184	250	94	3
	Cases	83	134	56	0
	Odds Ratio	1	1.196	1.306	
	95% CI		(0.852–1.679)	(0.857–1.992)	
	p-value		0.3	0.214	
Total	Controls	386	545	187	8
	Cases	194	290	104	3
	Odds Ratio	1	1.064	1.112	
	95% CI		(0.844–1.341)	(0.822–1.505)	
	p-value		0.599	0.491	