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Breast Cancer

Tamoxifen and CYP2D6: A Contradiction of Data

DANIEL L. HERTZ,[a,b] HOWARD L. MCLEOD,[a,b,c,d] WILLIAM J. IRVIN JR[b,c,d]

[a]UNC Eshelman School of Pharmacy, Division of Pharmacotherapy and Experimental Therapeutics, [b]Institute for Pharmacogenomics and Individualized Therapy, and [c]Department of Medicine, Division of Hematology-Oncology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; [d]UNC Lineberger Comprehensive Cancer Center, Chapel Hill, North Carolina, USA

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LEARNING OBJECTIVES

After completing this course, the reader will be able to:

1. Describe the significant heterogeneity among the published studies on the link between *CYP2D6* genotype and tamoxifen treatment efficacy.
2. Explain the role of CYP2D6 metabolism in the conversion of tamoxifen to its active metabolite, endoxifen, and the potential importance of *CYP2D6* polymorphisms to this process.
3. Discuss the role that insufficient genotyping, CYP2D6 inhibition, and tamoxifen combination treatment may have had in the inconsistent findings of past pharmacogenetic studies.

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ABSTRACT

Tamoxifen is an effective antiestrogen used in the treatment of hormone receptor–positive breast cancer. Bioconversion of tamoxifen to endoxifen, its most abundant active metabolite, is primarily dependent on the activity of cytochrome P450 2D6 (CYP2D6), which is highly polymorphic. Over 20 published studies have reported on the potential association between *CYP2D6* polymorphism and tamoxifen treatment outcome, with highly inconsistent results. The purpose of this review is to explore differences among 17 independent studies to identify factors that may have contributed to the discrepant findings. This report discusses six putative factors that are grouped into two categories: (a) clinical management criteria: hormone receptor classification, menopausal status, and tamoxifen combination therapy; (b) pharmacologic criteria: genotyping comprehensiveness, CYP2D6 inhibitor coadministration, and tamoxifen adherence. Comparison of these factors between the positive and negative studies suggests that tamoxifen combination therapy, genotyping comprehensiveness, and CYP2D6 inhibitor coadministration may account for some of the contradictory results. Future association studies on the link between *CYP2D6* genotype and tamoxifen treatment efficacy should account for combination therapy and CYP2D6 inhibition, and interrogate as many *CYP2D6* alleles as possible. *The Oncologist* 2012;17:620–630

Correspondence: William J. Irvin Jr., M.D., Physicians Office Building, 170 Manning Drive, 3rd Floor, Chapel Hill, North Carolina 27599-7305, USA. Telephone: 919-843-6616; Fax: 919-966-6735; e-mail: william_irvin@med.unc.edu Received November 30, 2011; accepted for publication March 8, 2012; first published online in *The Oncologist Express* on April 24, 2012. 1083-7159/2012/$20.00/0 http://dx.doi.org/10.1634/theoncologist.2011-0418

Introduction

Use of the antiestrogen tamoxifen for 5 years in hormone receptor $(HR)^+$ breast cancer is associated with a nearly 50% lower recurrence rate and provides an overall survival benefit [1]. However, ~20%—30% of women relapse despite the full 5 years of therapy [2]. Another class of endocrine therapy, the aromatase inhibitors, is slightly more effective than tamoxifen alone in the postmenopausal setting but is not appropriate for premenopausal patients [3, 4]. Aromatase inhibitors are not available or are prohibitively expensive in many parts of the world, with tamoxifen therefore being the primary choice for endocrine therapy for most patients.

The parent tamoxifen molecule has two primary metabolites: 4-hydroxy-tamoxifen and N-desmethyl-tamoxifen. Each metabolite can be bioconverted to (Z)-4-hydroxy-N-desmethyl-tamoxifen, commonly referred to as endoxifen (Fig. 1). Comprehensive analysis of tamoxifen and 22 of its metabolites confirms that endoxifen is the most abundant active metabolite of tamoxifen [5, 6]. Bioactivation of tamoxifen to endoxifen is mediated by a multitude of cytochrome P450 (CYP) enzymes, with CYP2D6 being central to metabolic activation [7, 8]. CYP2D6 is a highly polymorphic enzyme with >80 annotated isoforms [9]. These isozymes range in activity from splice variants with no metabolic capability to gene duplications that possess activity 10- to 30-fold greater than that of the wild-type enzyme [10]. Knowledge of *CYP2D6* genotype enables classification as a poor metabolizer (PM), intermediate metabolizer (IM), extensive metabolizer (EM), or ultrarapid metabolizer (UM), indicating the extent of drug metabolism [11].

More than 20 reports investigating whether or not *CYP2D6* genotype influences the efficacy of tamoxifen treatment have been published. The conclusions of these studies range from a possible longer disease-free survival interval [12] to a substantially shorter recurrence-free survival time [13] for patients carrying *CYP2D6* genotypes conferring diminished tamoxifen metabolism. These conflicting findings have led to confusion among clinicians and regulatory bodies regarding whether or not *CYP2D6* genotyping should be performed, and from a clinical standpoint, whether or not genotype-guided tamoxifen therapy should be pursued. The inconsistency in study results is likely attributable to heterogeneity in study designs and patient populations.

This review examines six factors that may have influenced the conclusions of CYP2D6–tamoxifen association studies. These factors are grouped into two categories: (a) clinical management criteria: HR classification, menopausal status, and tamoxifen combination therapy; (b) pharmacologic criteria: genotyping comprehensiveness, CYP2D6 inhibitor coadministration, and tamoxifen adherence. This process will inform some general interpretive rules around the CYP2D6–tamoxifen literature.

Studies Included in Review

A PubMed search was conducted using combinations of the following search terms: CYP2D6, tamoxifen, pharmacogenetic, and pharmacogenomic. All published studies that investigated the association of *CYP2D6* genotype with the effectiveness of tamoxifen therapy in the treatment of nonmetastatic breast cancer were eligible for inclusion in this review. As of August 2011, 22 studies on the association of *CYP2D6* genotype and tamoxifen efficacy in nonmetastatic breast cancer treatment were published; however, in a number of cases, populations were reused in multiple publications. In these cases, only the most recent, thorough study was included in this analysis and the others were excluded. In the first instance, the study from Schroth et al. [14] was included because it had combined and added to the populations used in previous studies by Goetz et al. [15–17] and Schroth et al. [18]. Similarly, Kiyotani et al. [13] reused a population from a previous study [19]. After removing this redundancy, there were a total of 17 independent studies published, six of which found an association between PM *CYP2D6* genotypes and inferior tamoxifen efficacy in nonmetastatic breast cancer treatment (positive), nine that found no relationship (negative), and two that reported the reverse association (opposite). All 17 studies included in the review are listed in Table 1 by classification (positive, negative, or opposite), with the country in which they were conducted, tamoxifen dose and duration, duration of follow-up, and clinical endpoints analyzed. For the purposes of comparison in this report, the nine negative and two opposite studies are combined into a single (negative) group.

Clinical Management Criteria

Retrospective studies are inherently confined to the population that was enrolled in the parent study or that existed in the clinical database from which it sampled. There are a variety of differences among the patient cohorts in these studies, including: patient enrollment method; percentage of total cohort available for pharmacogenetic testing; tumor stage and receptor status; patient race, age, and menopausal status; tamoxifen indication, dose, and duration; auxiliary treatment; follow-up duration; and measure of treatment efficacy. The relationship between *CYP2D6* genotype and tamoxifen efficacy may only exist in a subset of patients; therefore, any of these factors may be critical in determining whether or not the relationship, if it exists, could be detected in a given study. Three factors were selected to illustrate putative explanations for the discrepant reports: HR classification, menopausal status, and tamoxifen combination therapy.

HR Classification

Tamoxifen efficacy is limited to estrogen-dependent tumors, which express the estrogen receptor (ER) and/or progesterone receptor (PR) [1, 20]. However, some of the studies included ER^-PR^- patients, who do not benefit from tamoxifen, and therefore would not have differential benefit based on CYP2D6 activity. It has been suggested that a lack of centralized testing of HR status can lead to misclassification of participants within a study [21], and this was confirmed retrospectively [22]. Instead of conjecture on possible misclassifications, it may be informative to go through the positive and negative studies to identify which ones could have been influenced by the inclusion of HR^- tumors.

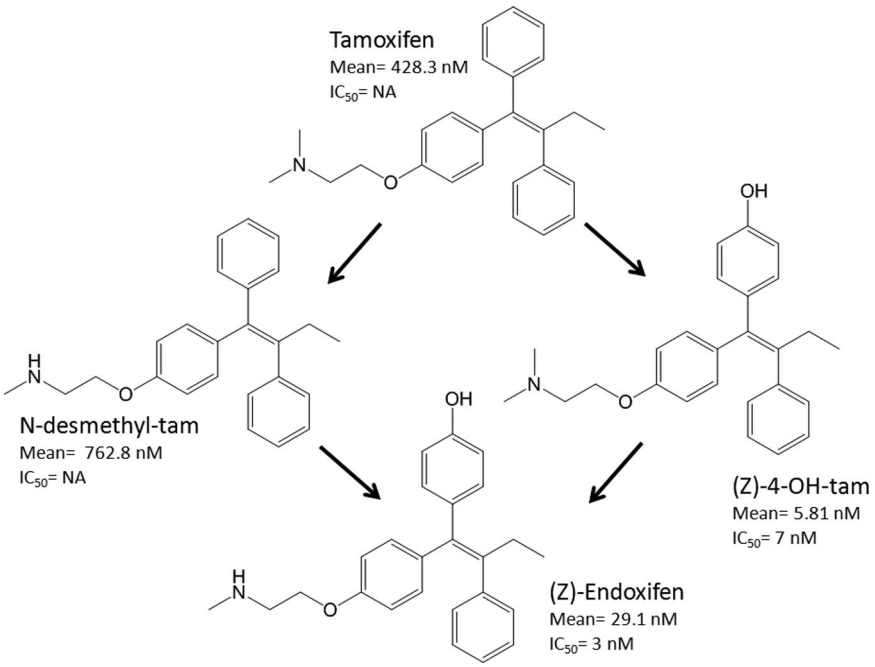

Figure 1. Tamoxifen metabolism. The metabolism of tamoxifen to endoxifen is heavily dependent on cytochrome P450 2D6 activity. The mean steady-state plasma concentrations and 50% inhibitory concentration (IC_{50}) values for each molecule [6] suggest that endoxifen is the active metabolite responsible for tamoxifen efficacy.

Abbreviations: 4-OH-tam, 4-hydroxy-tamoxifen; N-desmethyl-tam, N-desmethyl-tamoxifen; NA, not available.

Differences in reporting methods complicate this comparison. Table 2 lists the percentage of patients from each study who are ER^- and denotes which studies specified that all participants were HR^+. Contrary to what was expected, a slightly higher proportion of positive studies included ER^- patients. Excluding studies in which all patients were known to be HR^+ and the study with no known receptor status, 66% of the positive studies (two of three) and 50% of the negative studies (four of eight) included ER^- patients. In the four negative studies, the percentage of patients who were ER^- was low (5%–14%), so it is unlikely that this contributed to the negative findings, particularly in the study from Lash et al. [23], in which a subanalysis in ER^+ patients was also negative. The study with the largest percentage of ER^- patients overall (23%), from Newman et al. [24], reported a positive finding in the *BRCA2* mutation cohort (91% ER^+) but not in the *BRCA1* mutation cohort (57.5% ER^+); however, a pooled analysis of all ER^+ participants was not statistically significant.

The inclusion of HR^- individuals does not explain negative study findings or differentiate between positive and negative studies. Nevertheless, given what is known about tamoxifen's mechanism and efficacy, it is important that future studies confine their population to only HR^+ patients, in whom tamoxifen is effective at preventing cancer recurrence, and consider covariate adjustment for the ER^+PR^- and ER^-PR^+ subgroups, because a recent publication suggests that, given the ER status of a tumor, PR status is not an important predictor of tamoxifen efficacy [1]. Future research examining adjustment for different intrinsic subtypes of breast cancer within ER^+ tumors (i.e., luminal A versus luminal B) would also be illuminating in developing further predictive models for tamoxifen efficacy.

Menopausal Status

There has been speculation that the association of CYP2D6 and tamoxifen efficacy may be confined to premenopausal patients. In postmenopausal women, tamoxifen and N-desmethyl-tamoxifen together occupy >99.9% of the available ERs, suggesting that variation in endoxifen concentration would have little role in blocking estrogen signaling [25]. However, endoxifen may be critical to saturate the ER in premenopausal women, in whom tamoxifen and N-desmethyl-tamoxifen are estimated to occupy only 90%–95% of the available receptors [26]. If receptor occupancy, competition with estradiol, or some other mechanism causes endoxifen to be critical only in premenopausal women, then only studies that include primarily premenopausal patients would detect a CYP2D6–tamoxifen association.

Each of the 17 studies reported some measure of menopausal status or age, though differences in reporting methods make direct comparison among all studies impossible (Table 3). Looking at either the percentage of participants who were premenopausal, the average age of the patients, or the percentage of patients aged <50 years, there is little evidence that negative study results can be attributed to the enrollment of postmenopausal patients. On the contrary, studies with the highest percentages of premenopausal patients [27, 28] and patients aged <50 years [29] were negative whereas the study with the highest average age was positive [30]. Future studies should consider adjusting for menopausal status in case there is

Table 1. Studies included in review

Study findings	Study	Study country	Dose, mg/day	Duration of therapy, yrs	Duration of follow-up, yrs	Clinical endpoints
Opposite	Wegman et al. (2005) [59]	Sweden	40	2	Mean, 10.7; range, 0.24–18.6	DRecFS
	Wegman et al. (2007) [12]	Sweden	20 or 40	2 or 5	Median, 7.1; range, 0.04–17.9	RecFS
Negative	Nowell et al. (2005) [29]	USA	NR	NR	Median, 5.4; range, 3–14	PFS, OS
	Okishiro et al. (2009) [28]	Japan	20	Median, 4.3; range, 0.75–5	Median, 4.7; range, 0.67–9.1	RecFS
	Toyama et al. (2009) [65]	Japan	20	Median, 3.2; range, 2–5	Median, 7.9; range, 2.1–21	DFS, DDFS, BCSS, OS
	Stingl et al. (2010) [66]	Austria	20	Mean, 3.4; SD, 1.5	Mean, 7; SD, 1.6	TTP, PFS
	Ramón et al. (2010) [67]	Spain	NR	NR	Median, 9; range, 7.6–11	DFS
	Abraham et al. (2010) [32]	UK	20	NR	Mean, 6.0	BCSS, OS
	Kiyotani et al. (2010) [27]	Japan	NR	NR	NR	RecFS
	Lash et al. (2011) [23]	Denmark	20	1, 2, or 5[c]	Range, 1–10	BCRec
	Park et al. (2012) [60]	Korea	20	Median, 4.4; range, 0.5–5.9	Median, 5.6; range, 0.6–10	RecFS
Positive	Goetz et al. (2007) [16][a]	USA	20	5	Median, 11; range, 5.7–14	TTRec, RecFS, DFS, OS
	Schroth et al. (2007) [18][a]	Germany	NR	NR	Median, 6.4; range, 0.68–19	RFT, EFS, OS
	Xu et al. (2008) [45]	China	20	5	Median, 5.3; range, 0.33–10	DFS, DSS
	Newman et al. (2008) [24]	UK	20	Median, >4	Median, 10	TTRec, RecFS, OS
	Bijl et al. (2009) [30]	Netherlands	20 or 40	Mean, 2.13 SD, 1.8	NR	BCM, CM, M
	Schroth et al. (2009) [14][b]	Germany and USA	NR	5	Median, 6.3; range, 0.18–20	TTRec, DFS, EFS, OS
	Thompson et al. (2011) [31]	England	20	5	Median: cohort 1, 4.9; cohort 2, 9.4	RFS
	Kiyotani et al. (2010) [13]	Japan	20	5	Median, 7.1; range, 0.8–24	RecFS

[a]Not counted as an independent study because of inclusion of population in a later study.
[b]Includes previously reported populations.
[c]Prescribed duration: 1 (46%), 2 (18%), or 5 (36%) years; however, most patients were treated longer.
Abbreviations: BCM, breast cancer mortality; BCRec, breast cancer recurrence; BCSS, breast cancer–specific survival; CM, cancer mortality; DDFS, distant disease-free survival; DFS, disease-free survival; DRecFS, distant recurrence-free survival; DSS, disease-specific survival; EFS, event-free survival; M, mortality; NR, not reported; OS, overall survival; PFS, progression-free survival; RecFS, recurrence-free survival; RFS, relapse-free survival; RFT, relapse-free time; SD, standard deviation; TTP, time to progression; TTRec, time to recurrence.

a differential effect of tamoxifen in these populations; however, based on a comparison of the available studies, there does not seem to be a substantial influence of menopausal status on the possible association between *CYP2D6* genotype and tamoxifen efficacy.

Tamoxifen Combination Therapy

Kiyotani et al. [27] published a study suggesting that the inclusion of patients on combination therapy could have contributed to the negative findings of previous analyses. They classified patients who received any additional therapy, including radia-

Table 2. ER classification

Findings	Study	ER^- (unknown)	Notes
Negative	Wegman et al. [59]	0%	
	Wegman et al. [12]	0%	
	Stingl et al. [66]	0%	
	Ramón et al. [67]	0%	
	Park et al. [60]	3% (1%)	All participants HR^+
	Toyama et al. [65]	3% (1%)	All participants HR^+
	Lash et al. [23]	5% (9%)	Subgroup analysis in ER^+ participants not significant
	Abraham et al. [32]	7% (30%)	
	Okishiro et al. [28]	9%	All participants HR^+
	Kiyotani et al. [27]	11% (5%)	
	Nowell et al. [29]	14%	Adjusted for HR status
Positive	Bijl et al. [30]	0% (100%)	HR status unknown for all patients
	Thompson et al. [31]	0%	
	Schroth et al. [14]	3%	All participants HR^+
	Xu et al. [45]	9% (9%)	Subgroup analysis in ER^+ participants significant
	Kiyotani et al. [13]	11% (6%)	All participants HR^+
	Newman et al. [24]	23%	Pooled analysis of ER^+ patients among $BRCA1^+$ and $BRCA2^+$ patients not significant

Abbreviations: ER, estrogen receptor; HR, hormone receptor.

Table 3. Menopausal status or age

Findings	Study	Premenopausal	Study	Average age	Study	<50 years old
Negative	Wegman et al. [59]	0%	Park et al. [60]	Median, 45	Nowell et al. [29]	42%
	Wegman et al. [12]	0%	Abraham et al. [32]	Median, 53		
	Lash et al. [23]	6%	Stingl et al. [66]	Mean, 59		
	Ramón et al. [67]	43%	Toyama et al. [65]	Mean, 59		
	Kiyotani et al. [27]	71%				
	Okishiro et al. [28]	78%				
Positive	Schroth et al. [14]	4%	Newman et al. [24]	Median, 41[a] and 44[b]	Xu et al. [45]	24%
	Thompson et al. [31]	19%	Bijl et al. [30]	Mean, 76		
	Kiyotani et al. [13]	44%				

[a] $BRCA1^+$ cohort (negative association).
[b] $BRCA2^+$ cohort (positive association).

tion, as combined therapy. For our comparison, patients not receiving additional systemic therapy, excluding radiation, were classified as receiving tamoxifen monotherapy. Updating the original comparison of Kiyotani et al. [27], the use of additional therapy seems to differ between negative and positive studies (Table 4). Of the negative studies, only one used a cohort that received tamoxifen monotherapy (one of 11, 9%), compared with three of the five positive studies (60%). The study from Bijl et al. [30], which has no information on additional treatment, was excluded from this comparison. In fact, the nine studies with the lowest percentages of patients on monotherapy were all negative, whereas five of the seven studies with >70% of patients on monotherapy were positive. Additionally, one recent study showed a stronger association in the tamoxifen monotherapy subgroup [31]. However, the lack of association in the tamoxifen monotherapy subgroup in some recent negative studies [23, 28, 32] indicates that this single factor does not fully explain the conflicting findings. Never-

Table 4. Tamoxifen combination therapy

Findings	Study	Monotherapy[a]	Chemotherapy	Hormone therapy	Unknown Therapy	Note
Negative	Kiyotani et al. [27]	0%	67%	38%	7%	Some concurrent chemotherapy and hormonal therapy
	Park et al. [60]	22%	78%			
	Ramón et al. [67]	40%	60%			
	Okishiro et al. [28]	42%	24%	34%		Subgroup analysis in monotherapy patients NS
	Abraham et al. [32][b]	48%	19%		33%	Subgroup analysis in monotherapy patients NS
	Wegman et al. [59]	≈50%	≈50%			Patients randomized to chemotherapy or radiation
	Nowell et al. [29]	52%	48%			
	Stingl et al. [66]	58%	29%	13%		
	Wegman et al. [12][c]	≥65%	≤35%			
	Lash et al. [23]	88%	12%			Subgroup analysis in monotherapy patients NS
	Toyama et al. [65]	100%				
Positive	Newman et al. [24]	71%	28%			
	Thompson et al. [31]	80%	18%			Significant results in monotherapy patients
	Schroth et al. [14]	100%				
	Kiyotani et al. [13]	100%				
	Xu et al. [45]	100%				
	Bijl et al. [30]	Not reported			100%	

[a]Monotherapy defined as no systemic therapy (radiation excluded).
[b]Self-reported retrospectively.
[c]Calculated from prior publication [68].
Abbreviation: NS, not significant.

theless, it does seem that the influence of *CYP2D6* genotype on tamoxifen efficacy may be confounded by additional therapy, and only patients on tamoxifen monotherapy should be included in future studies of this potential pharmacogenetic association.

Pharmacologic Criteria

The theoretical association between *CYP2D6* genotype and tamoxifen efficacy relies on two assumptions. The first is that the *CYP2D6* genotype modulates endoxifen formation and can be used as a proxy for endoxifen exposure. This has been consistently demonstrated by many different groups [13, 33–35], with *CYP2D6* genotype explaining up to 40% of the variability in endoxifen steady-state concentrations [6]. The second assumption, that exposure to endoxifen dictates response, was not formally tested until a recent report from Madlensky et al. [36] demonstrated that individuals in the lowest endoxifen concentration group experienced an inferior benefit from tamoxifen therapy. Taken together, the data strongly suggest that *CYP2D6* genotype, through its influence on endoxifen biotransformation, could be an important predictor of tamoxifen efficacy.

Additional research suggests that endoxifen exposure can be modulated by a host of factors beyond CYP2D6 intrinsic function, leading to the development of elaborate phenotypic classification systems [37]. The coadministration of CYP2D6 inhibitors and the adherence to tamoxifen therapy influence the endoxifen concentration, and potentially tamoxifen efficacy [38, 39]. Thus, comparing the genotyping comprehensiveness, inhibitor coadministration, and tamoxifen adherence in the positive and negative studies may explain some of the inconsistency in the results of the 17 studies.

Genotyping Comprehensiveness

The original studies of *CYP2D6* genotype and tamoxifen efficacy focused exclusively on the *CYP2D6*4* allele, a splicing defect that produces an enzyme with no metabolic capacity. Additional alleles that affected metabolic activity are known, including the null activity *CYP2D6*3*, *CYP2D6*5*, and *CYP2D6*6* alleles, and reduced activity *CYP2D6*10* and *CYP2D6*41* alleles. For a more detailed review of the polymorphisms and their effect on activity see Zanger et al. [11]. The current gold standard for comprehensive *CYP2D6* genotyping is the U.S. Food and Drug Administration–approved

AmpliChip™ CYP450 Test (Roche Diagnostics, Indianapolis, IN), which interrogates 29 polymorphisms and copy number variants, enabling detection and categorization of 33 alleles as null, diminished, normal, or high activity [40].

The negative results of some studies may be attributed to insufficiently comprehensive genotyping, as demonstrated by Schroth et al. [41]. That paper compared the hazard ratio, study power, and percentage of individuals who would be classified as PM, IM, or EM in situations of varying numbers of genotyped alleles. Using the data from their clinical cohort, expanding the allelic coverage from only the *CYP2D6*4* allele to inclusion of all alleles on the AmpliChip™, increased the estimated hazard ratio from 1.33 ($p = .58$) to 2.87 ($p = .006$), with a corresponding increase in study power from 7.8% to 63.2%. Interestingly, although the percentage of PM patients had an absolute increase of only 2.8% (5.5% to 8.3%), there were dramatic changes in the percentage of IM (32.7% to 54.1%) and EM (61.8% to 37.6%) patients, suggesting that most studies misclassify many IMs as EMs. This clearly demonstrates that insufficiently comprehensive genotyping leads to patient misclassification and diminishes the ability to detect a genotype–outcome association.

In comparing the genotyping comprehensiveness between the positive and negative studies, there is a clear trend toward a greater likelihood of a positive finding with expanded allelic coverage (Table 5). Of the studies that interrogated a single variant, only 22% (two of nine) were positive. This percentage is higher, at 50%, for studies that assayed three to seven variants (three of six) or used the AmpliChip™ (one of two). In agreement with the findings of Schroth et al. [41], Thompson et al. [31] reported that their positive association would not have been detectable if they had exclusively genotyped the *CYP2D6*4* allele.

There is strong evidence that inadequate allelic coverage can diminish study power through patient misclassification. The negative findings in many, but not all, of these 17 studies may be attributed to insufficiently comprehensive genotyping. In the future, all studies should use a genotyping technology that is at least as comprehensive as the widely available AmpliChip™. Additional research should be undertaken to discover and characterize additional *CYP2D6* polymorphisms that influence tamoxifen-to-endoxifen bioconversion.

CYP2D6 Inhibitor Coadministration

Selective serotonin reuptake inhibitor (SSRI) antidepressants diminish the severity and occurrence of hot flashes, one of the most common adverse effects of tamoxifen [42]. Most SSRIs inhibit CYP2D6 [43], and coadministration with tamoxifen leads to lower concentrations of endoxifen [34, 44], an effect that may be most pronounced with the strong inhibitor paroxetine [38]. In an effort to clarify the relative importance of genetics and CYP2D6 inhibition, a recent study used information on *CYP2D6* genotype and inhibitor coadministration to develop a phenotype score to explain variability in the endoxifen/N-desmethyl-tamoxifen ratio. Coadministration of a CYP2D6 inhibitor explained 38%–53% of the variability whereas the genetic information provided no additional explanatory value [37].

If concomitant administration of CYP2D6 inhibitors diminishes endoxifen production, as demonstrated by previous data, then it is likely to abrogate tamoxifen efficacy as well. Therefore, it may be revealing to investigate which of the positive and negative pharmacogenetic studies accounted for CYP2D6 inhibition. One positive and one negative study excluded patients taking paroxetine or other strong CYP2D6 inhibitors [28, 45]. Of the remaining studies, a larger percentage of the positive studies (three of five, 60%) than the negative studies (two of 10, 20%) reported an assessment of inhibitor use in their analysis (Table 6). Additionally, the positive studies did a superior job of limiting their analysis to strong CYP2D6 inhibitors. The most commonly administered SSRI in the Dutch cohort used in the negative study from Lash et al. [23] is the weak inhibitor/noninhibitor citalopram, which may not interfere with endoxifen formation [46]. Similarly, the list of study medications classified as inhibitors by Abraham et al. [32] includes many medications that are merely substrates, severely limiting the relevance of this analysis.

Among the three positive studies that accounted for inhibitor use, only the study by Newman et al. [24] reported a significant effect on tamoxifen treatment outcomes. It should also be noted that one of the earlier studies from Goetz et al. [16], which was excluded because of population overlap with Schroth et al. [14], did include CYP2D6 inhibitors and reported better *p*-values after reclassification of patients based on inhibitor use.

The evidence presented in this review suggests that inhibitor coadministration may be influencing the results of pharmacogenetic studies. The theoretical concern of coadministration of strong CYP2D6 inhibitors during tamoxifen therapy has gained acceptance in clinical treatment and it would be ideal if future pharmacogenetic studies excluded or accounted for concomitant CYP2D6 inhibitor treatment.

Tamoxifen Adherence

Patient nonadherence to treatment is a common issue in outpatient drug therapy. Taking ≥80% of the doses prescribed is a frequently used threshold for adherence. Using this threshold, only 77% of patients are adherent after 1 year of tamoxifen. This number gradually declines to 50% by year 4 [47], and differences in the propensity to discontinue tamoxifen treatment by race, age, and disease have been reported [48]. Further complicating the inclusion of adherence data is the tendency of patients to exaggerate when self-reporting, which has been demonstrated specifically for hormonal agents [49]. Importantly, poor adherence has been linked to a poor breast cancer event-free time [50] and survival time [51]. Thus, poor tamoxifen adherence leads to suboptimal treatment efficacy, but it is very difficult to account for compliance in clinical studies, which may be biasing these retrospective pharmacogenetic analyses.

Unfortunately, only one of these 17 studies considered drug adherence. Thompson et al. [31] used prescription data to calculate that 14% of their patients were <80% adherent.

Table 5. Genotyping comprehensiveness

Findings	Study	Number of SNPs in analysis	Alleles interrogated in analysis	Note
Negative	Okishiro et al. [28]	1	**10*	
	Toyama et al. [65]	1	**10*	
	Wegman et al. [59]	1	**4*	
	Stingl et al. [66]	1	**4*	
	Wegman et al. [12]	1	**4*	
	Nowell et al. [29]	1	**4*	**3* and **6* assayed but excluded
	Lash et al. [23]	1	**4*	Quantitative bias analysis
	Park et al. [60]	3	**5, *10, *41*	
	Abraham et al. [32]	6	**4, *5, *6, *9, *10, *41*	Tag SNPs included
	Kiyotani et al. [27]	6	**4, *5, *10,*21, *36, *41*	Gene duplications included
	Ramón et al. [67]	29	Roche AmpliChip™	Gene duplications included
Positive	Xu et al. [45]	1	**10*	
	Bijl et al. [30]	1	**4*	
	Newman et al. [24]	4	**3, *4, *5, *41*	
	Schroth et al. [14]	5	**3, *4, *5, *10, *41*	Gene duplications included
	Kiyotani et al. [13]	7	**4, *5, *10, *14, *21, *36, *41*	Gene duplications included
	Thompson et al. [31]	29	Roche AmpliChip™	Gene duplications included

Abbreviation: SNP, single nucleotide polymorphism.

Reassigning these individuals to the decreased metabolizer group and rerunning their primary analysis increased their estimated hazard ratio from 2.57 to 3.02. This suggests that ignoring adherence may be diluting the estimation of effect size in the other 16 studies, though this conclusion is based on very limited data. Nevertheless, it could be an important factor that has not been addressed in most retrospective studies, which should be included, when possible, in the analysis of any future studies.

There may also be interplay among these factors, making it difficult to deconvolute the role of each. Discontinuation of tamoxifen therapy is more common in EMs [52] and in women who are coadministered CYP2D6 inhibitors [50], presumably to treat hot flashes. These findings suggest that EMs may experience greater hot flashes, but additional studies have been unable to detect this association [53–55]. The confounding interaction of adverse events, inhibitor coadministration, and tamoxifen adherence is difficult to untangle from the data presently available.

Other Factors and Studies

Other Unexplored Factors

This review selected only six of the myriad differences among these 17 studies. Other factors that have been suggested were not explored, such as the percentage of patients from the parent study that were included in the pharmacogenetic substudy [56]. Perhaps exploring the differences in study design or methods of patient enrollment across studies may be informative, or looking at differences in the racial composition of studies and the allele frequencies in those populations may partly explain the discrepant results, particularly in light of the recent evidence that individuals of African descent are twice as likely to carry a lower metabolism allele [57]. Another intriguing difference is the use of the larger 40-mg tamoxifen dose in some studies [30, 58], including both of the studies that reported an opposite association [12, 59]. Perhaps this higher dose can overcome the diminished bioactivation in IMs and PMs [55]. Finally, patients in many studies were allowed to cross over or switch from the tamoxifen to the comparator arm after the study. This was the case in the study from Park et al. [60], in which 26% of the patients crossed over to an aromatase inhibitor. Perhaps this therapeutic switch is in some way abrogating the association of genotype and efficacy. Additional differences such as disease stage, endpoint definition, and length of follow-up time may further assist in explaining the inconsistent findings of these studies.

Unpublished Studies Presented at the San Antonio Breast Cancer Symposium

At the 2010 San Antonio Breast Cancer Symposium (SABCS), pharmacogenetic subanalyses of two large, well-designed studies in postmenopausal patients—the Arimidex, Tamoxifen, Alone or in Combination (ATAC) and the Breast International Group (BIG) 1–98/International Breast Cancer Study Group (IBCSG) 18–98 studies—were presented. The results of those studies did not support the hypothesis that patients with low activity *CYP2D6* genotypes

Table 6. CYP2D6 inhibitor coadministration

Finding	Study	CYP2D6 inhibitors included in analysis, listed by inhibition strength[a]	Effect in patients on inhibitors
Negative	Park et al. [60]	Not included in analysis	NA
	Wegman et al. [59]	Not included in analysis	NA
	Wegman et al. [12]	Not included in analysis	NA
	Stingl et al. [66]	Not included in analysis	NA
	Toyama et al. [65]	Not included in analysis	NA
	Nowell et al. [29]	Not included in analysis	NA
	Kiyotani et al. [27]	Not included in analysis	NA
	Ramón et al. [67]	Not included in analysis	NA
	Okishiro et al. [28]	Excluded participants taking paroxetine	NA
	Lash et al. [23]	**Strong:** paroxetine, fluoxetine; **moderate:** sertraline; **weak:** citalopram, escitalopram; **substrate:** fluvoxamine; **not listed:** zimeldine, alaproclate, etoperidone.	None
	Abraham et al. [32]	**Strong:** paroxetine, fluoxetine; **moderate:** sertraline, cimetidine; **weak:** nefazodone; **substrate:** fluvoxamine, venlafaxine, clomipramine, amitryiptyline, haloperidol, perphenazine, thioridazine. **Not listed:** fluphenazine.	None
Positive	Schroth et al. [14][b]	Not included in analysis	NA[b]
	Kiyotani et al. [13]	Not included in analysis	NA
	Xu et al. [45]	No inhibitors coadministered	NA
	Thompson et al. [31]	**Strong:** paroxetine, fluoxetine, quinidine, bupropion.	None
	Bijl et al. [30]	**Strong:** paroxetine, fluoxetine; **moderate:** sertraline, cimetidine, amiodarone.	None
	Newman et al. [24]	**Strong:** fluoxetine; **moderate:** thioridazine; **weak:** trazodone.	Earlier recurrence

[a]Inhibition strength based on Drug Interactions: Cytochrome P450 Drug Interaction Table [43].
[b]Reclassification of participants based on CYP2D6 inhibitor coadministration improved the *p*-values in the analysis of an earlier study from Goetz et al. [16] whose population is included in this study.
Abbreviation: NA, not applicable.

had worse outcomes from tamoxifen therapy [53, 61]. However, those studies are in contradiction to the findings of the currently unpublished Austrian Breast and Colorectal Cancer Study Group Study 8 presented at the 2008 SABCS [62]. All three studies used large populations of HR^+ postmenopausal patients treated with tamoxifen monotherapy. None of the studies employed the AmpliChip™ test or included gene duplication, so there is the potential for some patient misclassification; however, the lack of a trend in the negative studies suggests that more comprehensive genotyping would not have materially changed the findings. Of the three, only the ATAC study controlled for potent CYP2D6 inhibitors and none incorporated tamoxifen adherence in their presented analysis.

Metastatic Disease and Prevention

Four published studies have reported an association between *CYP2D6* genotype and tamoxifen effectiveness in the metastatic [33, 58] and prevention [63, 64] settings. These studies answered different clinical questions from the studies discussed previously in this report, and their relevance to the above discussions is debatable. However, three studies reported a statistically significant association between tamoxifen effectiveness and metabolizer status based on *CYP2D6* genotype. The positive prevention study, from Serrano et al. [63], used the AmpliChip™ test and found that, among tamoxifen-treated individuals, the PM genotype was found more commonly in cases than in controls (15% versus 1.5%; $p = .035$). However, a larger analysis of the National Surgical Adjuvant Breast and Bowel Project P1 and P2 trials from Goetz et al. [64] could not replicate these findings. The two studies in the metastatic setting, both positive, included exclusively HR+ patients, many of whom were on prior therapy for adjuvant or metastatic disease. The study from Lim et al. [33] genotyped only the *CYP2D6*10* allele and did not report inhibitor use, whereas the study from Lammers et al. [58] genotyped six alleles and reported that patients taking CYP2D6 inhibitors had a shorter time to progression and overall survival time than patients not coadministered an inhibitor. In the future, the

metastatic setting, with its smaller, shorter studies, may be the ideal setting in which to prospectively assess the influence of *CYP2D6* genotype on tamoxifen efficacy.

Conclusions and Recommendations

The biological rationale for the relationship between *CYP2D6* genotype and tamoxifen effectiveness is tantalizingly plausible, yet continued research has been unable to determine whether or not the association exists, let alone whether or not it is clinically meaningful. In this report, six factors were selected to compare across eleven negative and six positive studies. The number of positive studies suggests that there is some patient group that differentially benefits from tamoxifen based on CYP2D6 activity. Any study that includes patients outside this group, or misclassifies patients within this group, is liable to underestimate the effect of genotype on outcome, potentially leading to a false-negative finding (type I error). Therefore, it is not surprising that no single factor can consistently differentiate all the positive studies from the negative ones. Based on this comparison, studies that enrolled patients on tamoxifen monotherapy, genotyped the *CYP2D6* gene more comprehensively, and accounted for CYP2D6 inhibitor coadministration were more likely to have positive findings. On the other hand, patient menopausal status did not seem to influence the likelihood of a study returning a positive result. Surprisingly, it does not seem that the inclusion of patients with HR^- tumors explained the contradictory findings, and there were too few studies that accounted for treatment adherence to make any inference. From this review it seems that the best setting in which to detect a difference in outcome from tamoxifen therapy would be a cohort of ER^+ patients treated with tamoxifen monotherapy, using comprehensive genotyping with adjustment for CYP2D6 inhibitors and tamoxifen adherence. However, two of the largest studies to date that best fit these criteria, the ATAC and BIG 1–98 studies presented at the SABCS 2010, did not detect the expected association. Perhaps this is an indication that there is no true effect of *CYP2D6* genotype on tamoxifen outcome, or that the effect is so modest as to make it not clinically relevant. At this time, CYP2D6 testing does not meet evidence for routine clinical use, but further studies, potentially using the criteria defined in this review, to delineate its role in tamoxifen therapy are warranted.

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Author Contributions

Conception/Design: Daniel L. Hertz, Howard L. McLeod, William J. Irvin Jr
Collection and/or assembly of data: Daniel L. Hertz, William J. Irvin Jr
Data analysis and interpretation: Daniel L. Hertz, Howard L. McLeod, William J. Irvin Jr
Manuscript writing: Daniel L. Hertz, Howard L. McLeod, William J. Irvin Jr
Final approval of manuscript: Daniel L. Hertz, Howard L. McLeod, William J. Irvin Jr

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