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REVIEW

Impact of *CYP2D6* polymorphisms on endoxifen concentrations and breast cancer outcomes

GS Hwang, R Bhat, RD Crutchley and MV Trivedi

We investigated the impact of germline *CYP2D6* genotyping done using the non-tumor specimen on endoxifen concentrations and/or clinical outcomes in breast cancer (BC) patients treated with tamoxifen in published studies. We evaluated published data from 13 001 patients in 29 studies. Mean ± s.d. endoxifen concentrations were significantly lower in poor metabolizers (PM) versus extensive metabolizers (EM) (8.8 ± 7.2 versus 22.3 ± 11.8 ng ml^{-1}; $P < 0.05$). The PM status did not influence clinical outcomes in majority of the studies. However, only one study followed the Gaedigk activity scoring for phenotypic assignments, which predicted recurrence-free survival in CYP2D6 poor metabolizers. In two independent studies with 1676 patients, low endoxifen concentrations predicted poor BC-free survival. From our review of published data we found that standardization of *CYP2D6* genotype-phenotype classification is needed in order to ensure effective evaluation of associations between CYP2D6 polymorphisms and endoxifen concentrations and BC outcomes. Universal implementation of this standardization classification system should be a priority among researchers and laboratories. Furthermore, additional clinical research is warranted to determine whether patients with *CYP2D6* PM phenotypes or low endoxifen levels will have better clinical outcomes with increased tamoxifen dosing compared to standard dosing.

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INTRODUCTION

It is expected that 231 840 women will be diagnosed with invasive breast cancer (BC) in 2015–2016 in the United States.[1] About 80% of all invasive BCs express hormone receptors (HR), which include estrogen receptors (ER) and progesterone receptors.[2,3] All patients with HR-positive BC are treated with various types of endocrine therapy, which includes selective estrogen receptor modulator (SERM).[4] Tamoxifen is the most extensively used SERM, and is the most common agent used, especially, in premenopausal women for prevention and treatment of BC. Tamoxifen therapy reduces the risk of invasive and non-invasive BC in patients at high risk and also reduces recurrence in BC patients.[5–7]

Tamoxifen selectively targets the ER signaling and further inhibits estrogen genomic activity.[8] Tamoxifen is metabolized extensively in the liver via cytochrome (CYP) P450 to active metabolite, endoxifen, by two major pathways: *N*-demethylation and 4-hydroxylation. A major metabolic pathway is demethylation by CYP3A4/5 to N-desmethyltamoxifen, which is further oxidized by CYP2D6 to endoxifen (4-hydroxy-*N*-desmethyltamoxifen). This pathway contributes to 92% of tamoxifen metabolism.[9] Another metabolic pathway includes hydroxylation by CYP2D6 to 4-hydroxytamoxifen (4HT), which is then further metabolized by CYP3A4 to the active metabolite, endoxifen. While not many function-altering variants in CYP3A4/5 metabolism have been reported, CYP2D6 is a highly polymorphic enzyme with over 100 variants reported commonly in many patients.[10] Therefore, pharmacogenomics of *CYP2D6* seems to be one of the more significant determinants of tamoxifen bio-activation to endoxifen and its overall potential impact on efficacy outcomes in BC patients.

Although many studies have evaluated the role of *CYP2D6* polymorphisms on endoxifen concentrations as well as long-term clinical outcomes with tamoxifen, the results are conflicting.[7] Retrospective analysis of two large prospectively conducted studies (ATAC and BIG 1-98) reported that *CYP2D6* genotype did not impact clinical outcomes with tamoxifen.[11,12] However, these studies used the tumor DNA for the *CYP2D6* genotyping. Several publications suggest that many BCs have gene deletions on chromosome 22 near the *CYP2D6* locus (22q13), resulting in a loss of heterozygosity, contributing to a significant deviation of the genotype distribution from Hardy-Weinberg equilibrium.[13–17] It is likely that this phenomenon may have skewed the accuracy of some of the studies accounting for specific *CYP2D6* genetic variations in BC patients. To overcome this issue, we conducted a systematic analysis of published clinical studies that have utilized normal/healthy tissue for *CYP2D6* analysis to describe the relationship between *CYP2D6* genotype, endoxifen concentrations, and BC outcomes. We have found that *CYP2D6* phenotype assigned based on the genotype in non-tumor tissues significantly impacts endoxifen concentrations, but its impact on BC outcomes is less clear. We also discuss here the clinical implications of these results and propose future directions for clinical research in this area.

MATERIALS AND METHODS

Search strategy

A systematic search of the literature via PUBMED was initially performed using the following expanded Medical Subject Headings (MESH) terms: 'tamoxifen', '*CYP2D6*', 'breast cancer', and

Department of Pharmacy Practice and Translational Research, University of Houston College of Pharmacy, Houston, TX, USA. Correspondence: Dr MV Trivedi, Associate Professor, Department of Pharmacy Practice and Translational Research, University of Houston, College of Pharmacy, 1441 Moursund Street, Houston, TX 77030 USA.
E-mail: mtrivedi@uh.edu
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'endoxifen' or 'recurrence' with the search limit 'title/abstract'. The search was conducted on 24 May, 2017 with no date restrictions. Additional reports were obtained from the references of the published papers and clinical trials related to the topic of the meta-analysis.

Selection criteria

The reports were selected after the abstracts were reviewed for the inclusion and exclusion criteria. Eligibility criteria for inclusion comprised of the following: full text articles, published in English, reporting either endoxifen concentrations for each phenotype and/or BC outcomes based on the analysis of *CYP2D6* genotype, use of non-tumor specimens, in premenopausal or postmenopausal women, with high risk of BC or patients with BC who were treated with tamoxifen 20 mg by mouth daily for at least 4 weeks. Studies reporting BC outcomes based on endoxifen levels were also included. Exclusion criteria were studies reporting duplicate results, studies performed using formalin-fixed paraffin-embedded (FFPE) tumor tissues, studies described only as abstracts or correspondence, and those using non-traditional tamoxifen dosing. Both prospective and retrospective studies were included. Each study was critically evaluated for validity based on consistency, accuracy, and balance between the groups, if applicable.

Data collection

From each study, we recorded the following information: sample size (*N*), stage of the BC, menopausal stage (and % of patients in each category), age, *CYP2D6* sample, *CYP2D6* analysis tool, endoxifen sample, time points for the endoxifen sample, endoxifen analysis tool, endoxifen concentrations for each *CYP2D6* phenotype classification, hot flash experience, concomitant medication usage and BC outcome (such as BC-specific survival (BCSS), recurrence-free survival (RFS), disease-free survival (DFS), progression-free survival (PFS), and/or overall survival (OS)). Since *CYP2D6* phenotype was determined based on its genotype using different criteria in various publications, the definition of poor metabolizer (PM), intermediate metabolizer (IM), extensive metabolizer (EM) and ultra-rapid metabolizer (UM) was recorded separately in Supplementary Table 1. The activity score was calculated for the genotypes considered in each phenotypic category to compare various studies based on the PharmGKB website for *CYP2D6* and tamoxifen (https://www.pharmgkb.org/guideline/PA166104966) and the criteria previously published.[18] Gaedigk activity score definition for *CYP2D6* was considered 0 for PM, 0.5 for IM, 1.0–2.0 for EM, and >2.0 for UM.[18,19] Endoxifen values were reported in ng ml^{-1}. For consistent reporting, nM values were multiplied by 0.3735 to obtain ng ml^{-1} for endoxifen concentrations.

Data analysis

To assess the relationship between the *CYP2D6* phenotype and endoxifen concentrations, only studies with documented endoxifen concentrations for any of the *CYP2D6* phenotypes were included. Studies with endoxifen concentration reported as mean ± standard deviation (s.d.) or 95% CI were included in the graphical representation. The difference in endoxifen concentrations between *CYP2D6* phenotypes were evaluated using one-way analysis of variance (ANOVA) analysis, followed by Dunnette's multiple comparison *post hoc* test. All the studies, even when the endoxifen values were reported as a median value, were tabulated and described. The association between *CYP2D6* phenotypes and BC outcomes were summarized using descriptive statistics as reported in individual studies.

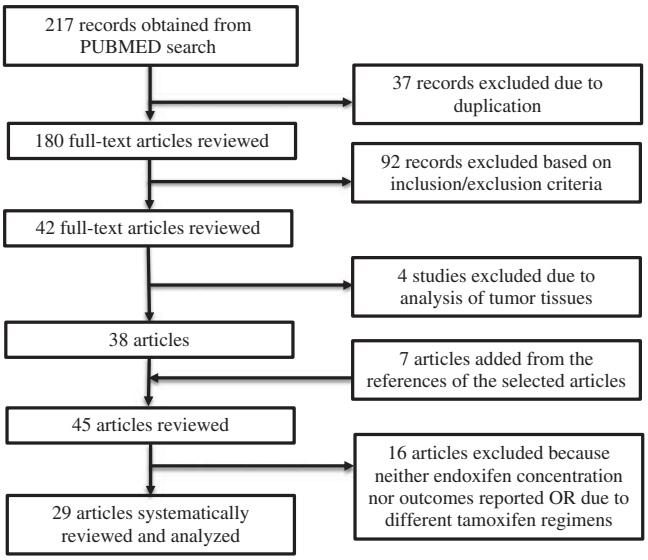

Figure 1. Flow chart of literature review process. The flow chart describes numbers of studies that are included in our analysis and the reasons other studies are excluded.

RESULTS

Study characteristics and patient demographics

Total of 217 records were obtained from PUBMED search. Figure 1 describes the selection of articles for this study. We evaluated the data for 13 001 patients from a total of 29 studies.

Detailed information on all the studies included in this review is provided in Table 1. Among the 29 articles,[20–48] 18 were prospective studies including cohort studies,[20–37] and three were case-control studies.[38–40] The other eight articles were retrospective studies.[41–48] Eighteen studies included patients in the early stage BC and on tamoxifen adjuvant therapy.[21,23,26–32,34,35,37,40,42,43,45,47,48] Two of the articles evaluated patients on tamoxifen for prevention purposes;[38,46] whereas one study was in the recurrence/metastatic setting.[41] Six studies included patients of all stages of cancer including metastatic stage.[20,24,33,36,39,44] Two studies did not report the stage of BC for the subjects enrolled in the study.[22,25] Overall, all studies included premenopausal or postmenopausal female patients with ER-positive BC at age of 18 or older. The activity scores calculated for each of the phenotypic categories as assigned by each of the studies are summarized in Table 1. Gaedigk activity score definition for *CYP2D6* was considered 0 for PM, 0.5 for IM, 1.0–2.0 for EM, and >2.0 for UM.[18,19] Out of 29 studies, 28 did not follow this standard definition of activity score to assign various genotypes in each phenotypic category (Table 1). In these 28 studies, most had an overlap in the activity scores for different CYP2D6 phenotypes as calculated in Table 1. Only one study reported BC outcomes using this activity scoring classification system.[24] Most studies reported if patients used any concomitant medications such as CYP2D6 inhibitors, since these may cause drug-drug interactions with tamoxifen therapy. However, detailed description in terms of their CYP2D6 inhibitory activity (strong, medium, weak) of these medications was not provided in most studies.

Association between *CYP2D6* phenotypes and endoxifen concentrations

A total of 11 studies reported endoxifen concentrations based on *CYP2D6* phenotypes. All these studies collected blood samples from subjects to determine *CYP2D6* genotypes and characteristic phenotypes. Only the studies with mean values ± s.d. reported

Table 1. Study characteristics

Cit	Study & patients (preM/postM/U)	TAM duration	N	Median age (yr)	DNA analysis		Activity score for genotype assignment for each phenotype				Endoxifen		Other medications (% of pts)	Statistical Significance	
					Source	Method	PM	IM	EM	UM	Sample	Assay		[Endo]	BC outcome
20	P; Stage I–IV BC (NR)	3– yrs	3155	53	B	Taqman SNP	0–0.5	1–1.5	2	NR		NR	CYP2D6i (6.1)	NR	No[a]
21	P; Stage I–II BC (56/44/0)	>90 d	117	53.3	B	Tag-It	0	1–1.5	2	NR	PL	LC–t-MS	CAM and CYP2D6i (13)	Yes	NR
22	P; NR (0/100/0)	>180 d	85	75.5	B	Taqman SNP	0	1	2	NR		NR	CYP2D6i (13)	NR	Yes
23	P; Stage I–III BC (NR)	4 mo	158	54	B	Taqman SNP	0	0.5–1	2	>2	PL	HPLC	CYP2D6i (29), Vit E (NR), SSRI (21), or CAM (NR)	Yes	NR
24	P; Stage I–IV BC (9/91/0)	>5 yrs	132	51	B	Taqman SNP	0	0.5	1–2	NR		NR	CYP2D6i (0)	NR	Yes
38	CC; Prevention (0/100/0)	5 yrs	591	59	B	Taqman SNP	0–0.5	0.5–2	2–3	3		NR	CYP2D6i (27)	NR	No
25	P; NR (56/44/0)	⩾4 mo	119	49	B	Amplichip	0	0.5–1.5	2	NR	PL	LC–t-MS	SSRI/SNRI (25), CYP2D6i (0)	Yes	NR
26	P; Early stage BC (43.6/53.8/3.6)	>4wks	282[b]	51	B	Taqman SNP	0–1.5	1–2.5	2	NR	PL	HPLC–TOFMS	SSRI (0)	Yes	Yes
27	P; Stage I–III BC (NR)	NR	1370	NR	B	Amplichip	0	1–⩾2	2	⩾3	S	HPLC	SSRI (NR)	Yes	NR[c]
28	P; Early stage BC (0/100/0)	6 mo	236	64.5	B	Taqman SNP	0	1	2	NR	PL	LC–t-MS	CYP2D6i (NR)	Yes	NR
29	P; Early stage BC (NR)	>2 mo	65	56.5	B	Amplichip	0	1	1–2	2	S	HPLC–MS/MS	MAOI & CYP2D6i (3)	Yes	NR
30	P; Stage I–III BC (NR)	>6 mo	716	45	B	SNaPshot	0–1	1–1.5	2	NR	PL	HPLC	NR	Yes	No
31	P; Stage I–III BC (32/38/29)	8–56 wks	99	50	B	Amplichip		NR			S	LC–t-MS	NR	Yes	NR
32	P; Stage I–III BC (100/0/0)	>1 yr	587	39.1	B	Taqman SNP		NR			PL	LC–t-MS	CYP2D6i, SSRI, SNRI, (NR)	NR	NR[c]
41	R; Stage IV & recurrent (NR)	>8 wks	202	47	B	SNaPshot	0–1	1–1.5	2	NR	PL	HPLC	CYP2D6i or inducers (0)	Yes	Yes
42	R; Stage I–III BC (NR)	>4 mo	224	NR	B	Taqman SNP	0.5–1.5	1.5	2	NR	S	LC–t-MS	NR	Yes	NR
43	R; Stage I–III BC (NR)	>4 yrs	115	NR	B	Taqman SNP	0	1	2	NR		NR	CYP2D6i (6.9)	NR	No
44	R; Stage I–IV (NR)	NR	165	NR	FFPE–nLN	Taqman SNP	0	1	2	>2		NR	NR	NR	No
45	R; Stage I–III BC (60.4/39.6/0)	NR	91	51	B	Amplichip	0–0.5	1–1.5	2–3	NR		NR	SSRIs (0)	NR	No
46	R; Prevention (NR)	>6 mo	265	NR	B	Amplichip		NR				NR	CYP2D6i (25% in EM)	NR	No
34	P; Stage I–III BC (22.4/76.7/1)	>1 yrs	313	60.2	B	Taqman SNP	0–0.5	1–1.5	1.5–2	3–4		NR	CYP2D6i (6.1)	NR	Yes
33	P; 0–IV BC (34.7/65.3/0)	NR	95	51	B	BioTools Taq	NR	<1	⩾1	NR		NR	NR	NR	Yes
36	P; I–IV BC (78/22/0)	>8 mo	173	47	B	Taqman SNP	1	1.5	2	NR		NR	Chemotherapy and/or goserelin	NR	No
48	R; I–III BC (62.5/37.5/0)	NR	48	51	B	Amplichip	NR	0.5–1	2	NR		NR	SSRI (NR)	NR	Yes
47	R; I–III BC (47.5/NR/NR)	>12 mo	99	48	B	Taqman SNP	0	0.5–1.5	2	>2		NR	Chemotherapy	NR	No
39	CC; I–IV BC (0/100/0)	NR	477	51	B	BeadChip SNP		NR				NR	NR	NR	No
37	P; I–III BC (NR)	NR	110	43.6	B	9700 Thermal Cycler		NR				NR	NR	NR	Yes
35	P; I–III BC (NR)	>9 mo	333	59.3	B	Taqman SNP	0	0.5–1.5	2	NR		NR	Strong CYPi (3.9) Intermediate CYPi (3.3)	NR	No
40	CC; I–III BC (79.5/20.5/0)	NR	39	46.12	B	Taqman SNP	1	1.5	2	NR		NR	NR	NR	Yes

Abbreviations: B, blood; BC, breast cancer; CAM, complementary and alternative medicine; CC, case–control; Cit, citation; CYP2D6i, CYP2D6 inhibitors; d, day; EM – extensive metabolizer; [Endo], endoxifen concentrations; FFPE–nLN, formalin fixed, paraffin embedded normal lymph node(s); HPLC, high-performance liquid chromatography; HPLC–MS/MS, high-performance liquid chromatography mass spectrometry; LC–t-MS, liquid chromatography tandem mass spectrometry; LN, lymph node; MAOI, monoamine oxidase inhibitor; mo, months; N, sample size; NR, not reported; P, prospective; preM, premenopausal/perimenopausal; PL, plasma; postM, postmenopausal; R, retrospective; S, serum; SNaPshot, SNaPshot Multiplex System; SNRI, serotonin–norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; Tag-It™, Tag-It trade mark Mutation Detection system; TAM, tamoxifen 20 mg daily; Taqman SNP, TaqMan single nucleotide polymorphism SNP genotyping; U, unknown; UPLC–MS, ultra-performance liquid chromatography tandem mass spectrometry; vit E, vitamin E; wks, weeks; yrs, years. [a]BC outcome was significantly poorer for patients with *CYP2D6* *6b or *6c alleles. [b]Among 282 total patients on tamoxifen, only 98 patients were evaluated for endoxifen concentrations, but all were genotyped and were assessed for breast cancer outcomes. [c]Although the clinical outcome in relationship to *CYP2D6* phenotype was not reported, endoxifen concentrations predicted clinical outcomes in these studies.

were considered in Figure 2,[23,27–31,41,42] while the studies with median value reported are included in Table 2 separately.[21,25,26] Mean endoxifen concentrations reported in 8 studies ($N = 2861$) were 2.5-fold lower in subjects who had *CYP2D6* PM phenotypes (8.8 ± 7.2 ng ml^{-1}) compared to those with *CYP2D6* EM phenotypes (22.3 ± 11.8 ng ml^{-1}; $P \leqslant 0.05$, One-way ANOVA followed by Dunnett's multiple comparison *post hoc* test). There were no significant differences between endoxifen concentrations in subjects who had *CYP2D6* IM phenotypes (15.7 ± 10.8 ng ml^{-1}) or UM phenotypes (20.8 ± 9.2 ng ml^{-1}) compared to the subjects with EM phenotypes. In the three studies reporting median values with range, the endoxifen concentrations numerically increased from PM to IM to EM to UM (Table 2).

Correlation between *CYP2D6* phenotypes and BC outcomes

A total of 20 studies reported BC outcomes in patients who had available information regarding *CYP2D6* phenotypes (Table 3). Eleven studies reported survival outcomes such as BCSS, RFS, DFS, or PFS,[20,24,26,30,36,37,43–46,48] out of which 3 studies also reported OS.[20,35,36] Seven additional studies reported BC recurrence or disease event.[25,33–35,39,43,46] Whereas, BC mortality (BCM),[22] time to progression (TTP),[41] and recurrence-free time[47]were reported by one study each. Among the 20 studies, only nine studies reported significant associations between patients who had *CYP2D6* PM phenotypes and a higher risk of BCM, higher recurrence rate, poorer RFS or OS, or faster TTP compared to those patients with *CYP2D6* EM phenotypes.[22,24,26,33,34,37,40,41,48] The remaining eleven studies found no significant associations between *CYP2D6* phenotypes and BC outcomes.[20,30,35,36,38,39,43–47] However, only one study[24] out of the 20 we reviewed followed the Gaedigk activity scoring for CYP2D6 phenotypic assignments, which reported poor recurrence-free survival in CYP2D6 poor metabolizers.

Correlation between endoxifen concentrations and BC outcomes

Two studies reported BC outcomes in relationship to endoxifen concentrations. The study by Madlensky *et al*.,[27] reported that among 1370 patients, those with endoxifen concentrations above 5.97 ng ml^{-1} (15.98 nM) had 30% lower risk of additional BC events (HR = 0.70, 95% confidence interval: 0.52–0.94). Another study by Saladores *et al*.[32] found that among 548 premenopausal hormone receptor-positive BC patients who were adherent to tamoxifen therapy, those with low (< 14 nM or < 5.2 ng ml^{-1}) compared with high (> 35 nM or > 13.1 ng ml^{-1}) endoxifen concentrations were associated with shorter distant relapse-free survival (univariate $P = 0.03$; multivariate HR = 1.94; 95% confidence interval: 1.04–4.14, $P = 0.064$).

Ongoing clinical trials

We also reviewed current ongoing clinical trials to determine if any additional information was available regarding our topic of study. An on-line search was done on 9 April, 2017 using the clinical trials website (clinicaltrials.gov) of US National Institutes of Health. Among the 22 studies matched with the terms 'tamoxifen,' 'breast cancer,' and '*CYP2D6*,' three were excluded due to termination or withdrawal of the study. Three studies were also excluded because *CYP2D6* genotypes of BC patients were not analyzed. All of the 16 relevant ongoing studies being conducted in pre- or postmenopausal adult BC patients are summarized in Table 4.

Among the 16 studies, 6 studies are interventional studies, whereas 10 studies are observational. Among the six interventional studies, four studies are investigating the impact of increasing tamoxifen dose (from 20 to 40 mg per day) on its metabolism in patients with PM or IM *CYP2D6* phenotypes (NCT01075802, NCT01192308, NCT00764322, NCT00963209). One study is investigating the incidence of invasive and non-invasive BC with 5 mg per day tamoxifen versus placebo in patients with ductal/lobular carcinoma-*in situ* (NCT01357772), and the other study is evaluating the safety and maximum tolerated dose (MTD) of Z-endoxifen (NCT01273168) in patients with metastatic BC. Among the 10 observational studies, the primary objective of 6 of these studies is to evaluate the effects of *CYP2D6* polymorphisms and/or phenotype on tamoxifen metabolism (NCT00717015, 01220076,00900744) or disease/progression-free survival (NCT00973037, 01181518, 01124695). The frequency of *CYP2D6* polymorphisms and phenotype and its association with outcomes is investigated in 2 additional studies (NCT01169792, 00830973). One study is investigating the effect of endoxifen concentrations on BC outcomes (NCT00815555), and another study is evaluating changes in tamoxifen metabolism when using concomitant administration of medications that are CYP2D6 inhibitors (NCT00667121). The latter two studies mentioned above are critical to determine the importance of using therapeutic drug monitoring for endoxifen concentrations to help guide appropriate tamoxifen dosing to ensure positive treatment outcomes. These studies may also be helpful to further define potential clinically relevant drug–drug interactions in patients using both tamoxifen and CYP2D6 inhibitors. However, all these studies are being conducted in relatively small number of patients, and additional larger clinical trials are necessary to better understand this clinically important issue.

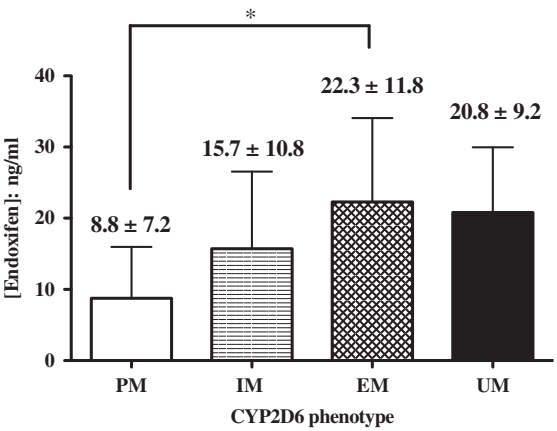

Figure 2. Endoxifen concentrations in patients with various *CYP2D6* phenotypes. The mean ± s.d. of endoxifen concentration (ng ml^{-1}) is plotted as a bar graph for each *CYP2D6* phenotype from 8 studies ($N = 2861$). * indicates statistically significant difference ($P < 0.05$, One-way ANOVA followed by Dunnette's multiple comparison test). Although different studies defined *CYP2D6* phenotypes differently, we found that mean endoxifen concentrations were significantly lower in the poor metabolizer (PM) group versus the extensive metabolizer (EM) group. There were no significant differences between mean endoxifen concentrations in the intermediate metabolizer (IM) or ultra-rapid metabolizer (UM) groups versus the EM group.

DISCUSSION

Our systematic analysis found that endoxifen concentrations were significantly lower in patients with PM phenotype for *CYP2D6* compared to EM phenotype. The difference in the endoxifen concentrations in the patients with the *CYP2D6* IM and UM phenotypes compared to EM phenotype were not statistically significant. The PM status did not impact clinical outcomes in majority of the studies. However, only one study followed the Gaedigk activity scoring for phenotypic assignments,[24] which reported poor RFS in poor metabolizers. In two independent studies with 1,676 patients, low endoxifen concentrations predicted poor BC-free survival. Below, we discuss the strengths

Table 2. Association between *CYP2D6* phenotypes and median endoxifen concentrations in patients receiving tamoxifen 20 mg once daily

Cit [a]	*Endoxifen concentration (ng ml^{-1})*											
	PM			*IM*			*EM*			*UM*		
	Median	*Range*	N	*Median*	*Range*	N	*Median*	*Range*	N	*Median*	*Range*	N
21	11.6	8.6–15.7	3	14.2	9.7–19.4	10	16.8	8.5–42.1	31	19.0	9.3–40.7	72
25	4.6	NR	11	19.8	NR	74	34.9	NR	32	NR	NR	NR
26	15.5	12–32.5	63	27.2	5–58	136	35.4	8–78	83	NR	NR	NR

Abbreviations: Cit, citation; EM, extensive metabolizer; IM, intermediate metabolizer; *N*, number; NR, not reported; PM, poor metabolizer; UM, ultrarapid metabolizer. [a]Different studies defined the *CYP2D6* phenotypes differently. See Supplementary Table 1 for further detail.

Table 3. Breast cancer outcomes based on *CYP2D6* phenotypes

Cit	*Breast cancer outcomes (confidence interval)*		
	PM	*IM*	*EM*
20	BCSS HR: 1.01 (0.8, 1.2)[a] OS HR: 1.05 (0.9, 1.3)[a]	BCSS HR: 0.93 (0.55, 1.57) OS HR: 0.98 (0.63, 1.54)	Ref
22	BCM HR: 4.1 (1.1, 15.9)[b]	BCM HR: 1.9 (0.9 – 3.9)	Ref
24	RFS OR: 12.37 (3.23, 47.33)[b]	NR	Ref
38	BCR OR: 0.942 (0.609, 1.458)	BCR OR: 0.957 (0.697, 1.315)	Ref
26	RFS HR: 9.52 (2.79, 32.45)[b]	RFS HR: 4.44 (1.31, 15.00)[b]	Ref
41	TTP: 5 mo[b]	NR	TTP: 21.8 mo.
43	R HR: 2.1 (0.84, 5.4) RFS HR: 1.9 (0.8, 4.8) OS HR: 2.5 (0.8, 8.2)	NR	Ref
44	PFS HR: 0.76 (0.33, 1.35); OS HR: 0.77 (0.32, 1.81)		Ref
30	RFS HR: 1.34 (0.4, 4.3)	Ref	
45	Mean DFS: 98 mo.	Mean DFS: 114 mo.	Mean DFS: 118 mo.
46	Disease event OR: 1.02 (0.31, 3.32)	Disease event OR: 0.81 (0.3, 2.23)	Ref
34	R HR: 0.39 (0.18, 0.85)[b, c] BCM HR: 0.33 (0.12, 0.9)[b, c]	NR	Ref
33	NR	R&M: 13.14 (1.54, 109.94)[b]	Ref
36	Median RFS: 63 mo	Median RFS: 54 mo	
48	NR	DFS: NR but not different from EM ($P = 0.273$); HR 6.85 (1.48–31.69)[b] in postmenopausal women; HR 10.52 (1.56–70.79)[b] in postmenopausal women who are homozygous for *10	Ref
47	RFT: NR but not different from EM and UM ($P = 0.19$)		Ref[d]
39	CBC RR: 1.2 (0.8, 1.7)	CBC RR: 0.8 (0.5, 1.5)	Ref
37	RFS HR: 5.59 (0.93,33.5) OS: $P = 0.01$[b]	RFS HR: 1.06 (0.21,5.25) OS: Not significant	Ref
35	BCE HR :0.50 (0.07–3.82)	BCE HR: 1.00 (0.47–2.11)	Ref
40	DFS: NR but not different from EM ($P = 0.316$)	DFS: NR but significantly different from EM ($P = 0.008$)[b]	Ref

Abbreviations: BCE, early breast cancer event; BCM, breast cancer mortality; BCR, breast cancer risk; BCSS, breast cancer-specific survival; CBC, contralateral breast cancer; Cit, citation; DFS, disease free survival; EM, extensive metabolizer; HR, hazard ratio; IM, intermediate metabolizer; mo., months; NR, not reported; OR, odds ratio; OS, overall survival; PFS, progression-free survival; PM, poor metabolizer; R, recurrence; M, metastasis; Ref, reference; RFS, recurrence free survival; RFT, relapse free time; RR, rate ratio; R&M, recurrence and metastasis; TTP, time to progression. [a]BC outcome was significantly poorer for patients with *CYP2D6* *6b or *6c alleles. [b]Indicates $P < 0.05$ (statistically significant). [c]A trend for significant difference in premenopausal women, but not in postmenopausal women in multivariate analysis. [d]Ref was EM+UM (extensive metabolizer or ultrarapid metabolizer) category.

and limitations of our analysis, discuss the potential reasons for discrepancies in various studies, and suggest future directions of clinical research in this area.

One of the challenges in understanding the role of *CYP2D6* is the source of tissue for its genotyping. Cancer in general, including BC, has significant chromosomal instability, accounting for loss of regions on various chromosomes. In the past, evaluation of FFPE tumor samples have led to misinterpretation of the data because of loss of heterozygosity in these samples and caused deviations from Hardy–Weinberg principles.[17] Therefore, using tumor tissues for genotyping may not be the best method to obtain accurate results. While the bias caused by discordant genotyping has been debated,[49] non-tumor tissues provide more suitable material for germline DNA analysis. In our study, only the studies assessing the germline rather than somatic mutations in *CYP2D6* were included to avoid this concern.

Lack of consensus between various studies evaluated here stem from various definitions for *CYP2D6* phenotype and differences in clinical characteristics. For example, some studies classified only *CYP2D6* *4/*4 as PM; whereas, other studies included other genotypes such as *CYP2D6* *3/*3, *3/*5, *6/*9, *4/*10, and *5/*41* in the PM category as well. As of now, there is no standardization of the *CYP2D6* genotype to phenotype classification. Interestingly, only one study out of 20 reporting the relationship between *CYP2D6* phenotype and BC outcomes followed the activity score guidelines adopted by Clinical Pharmacogenetics Implementation Consortium (CPIC) which was initially introduced by Gaedigk *et al*.[18] Because most studies did not follow the Gaedigk activity

Table 4. Summary of ongoing clinical trials

Trial number	*Study*	*Age (y)*	*Patients*	*Primary endpoint*	*Status*
NCT01075802	I	⩾ 18	121 early-stage, locally advanced or MBC patients	Effects of *CYP2D6* genotype and TAM dose↑ on [tamoxifen] and [its metabolites]	Completed
NCT01192308	I	⩾ 18	42 patients receiving adjuvant TAM	Effect of *CYP2D6*genotype and TAM dose↑ on [tamoxifen] and [its metabolites]	Completed
NCT00764322	I	⩾ 21	501 patients with BC or DCIS on TAM	Changes in [endoxifen] after TAM dose↑ in IM group	Unknown
NCT00963209	I	⩾ 18	140 BC patients on TAM	Impact of *CYP2D6* genotype and TAM dose↑ on its metabolism	Unknown
NCT01357772	I	18–75	1400 patients with LCIS or DCIS	Incidence of invasive and non-invasive BC with 5 mg/d TAM vs. placebo	Active, not recruiting
NCT01273168	I	18–120	72 MBC patients	Safety and MTD of Z-endoxifen	Recruiting
NCT00717015	O	⩾ 18	200 patients receiving adjuvant TAM	Effect of *CYP2D6* polymorphism on [tamoxifen] and [its metabolites]	Completed
NCT01220076	O	⩾ 18	265 BC patients on pre-operative TAM	*CYP2D6* genotype and TAM metabolism	Recruiting
NCT00900744	O	⩾ 18	100 early-stage BC patients on TAM	*CYP2D6* genotype and [endoxifen]	Unknown
NCT00973037	O	18–45	Early-stage BC	Impact of *CYP2D6* genotype on DFS	Unknown
NCT01181518	O	⩾ 18	1000 Asian BC patients	Association between polymorphisms of *CYP2D6/ CYP2C19/ CYP3A5* and DFS	Unknown
NCT01124695	O	⩾ 18	240 MBC patients on TAM monotherapy	Impact of *CYP2D6* phenotype score on PFS	Unknown
NCT01169792	O	⩾ 18	BC patients on adjuvant TAM	Frequency of *CYP2D6/ CYP2C19/ CYP3A4/5* polymorphisms and its association with outcomes	Completed
NCT00830973	O	⩾ 50	184 patients receiving TAM for BC prevention	Prevalence poor metabolizer status of *CYP2D6*	Completed
NCT00815555	O	⩾18	200–300 BC patients on adjuvant TAM	[Endoxifen] and BC relapse in patients with *CYP2D6 *4/*4* genotype (PM phenotype)	Unknown
NCT00667121	O	18–120	85 patients receiving TAM and a CYP2D6i	Change in [tamoxifen] and [endoxifen] after concomitant administration with CYP2D6 inhibitor	Active, not recruiting

Abbreviation: BC, breast cancer; CYP2D6i, CYP2D6 inhibitor; DCIS, ductal carcinoma *in situ*; I, interventional; IM, intermediate metabolizer; LCIS, lobular carcinoma *in situ*; MBC, metastatic breast cancer; MTD, maximum therapeutic dose; O, observational; PFS, progression free survival; PM, poor metabolizer; TAM, tamoxifen; □, concentration; ↑, dose elevation; Z-endoxifen, Z-isomer of endoxifen.

score classification system, it is possible that discrepancies between studies can be accounted for by variable definitions used to determine CYP2D6 phenotypes. This information reflects a common, unmet clinical need to standardize the genotype–phenotype classification for *CYP2D6* and ensure adequate education of researchers and laboratories conducting genetic testing so that proper assignment of CYP2D6 phenotypes is successfully implemented.

Another source of discrepancy could be the smaller sample size in most of the studies impacting the results, especially, considering the highly polymorphic nature of *CYP2D6*. Moreover, some studies tested many different alleles; whereas, others had only 2 (**1* and **4*) for assignment of the phenotype. The testing of fewer alleles could also result in more patients with *CYP2D6* genetic alterations being categorized in the EM group by default. In addition, all studies did not have a consensus on patient usage of concomitant medications. While some studies excluded the patients using CYP2D6 inhibitors or selective serotonin reuptake inhibitors (SSRIs), other studies included information regarding patient usage of these medications. Knowledge of concomitant medications that are strong CYP2D6 inhibitors is important since these can affect the metabolism of tamoxifen, as well as resulting endoxifen concentrations. For example, patients who might have a CYP2D6 EM phenotype and are concomitantly taking strong CYP2D6 inhibitors will essentially have a phenotype conversion to a CYP2D6 PM phenotype. Therefore, specific knowledge of drug–drug interactions and the degree of inhibition of concomitant medications is necessary to fully determine its overall impact on the appropriate use of tamoxifen therapy, its disposition, and related clinical outcomes.

Patient-specific factors may also influence tamoxifen metabolism; it is possible that these were not controlled for among the different studies. Because tamoxifen is a competitive antagonist for estrogen receptors, the levels of estrogen will impact the potency of tamoxifen, and hence would be relevant at least from the efficacy perspective. For example, one study found an association between reduced CYP2D6 activity and recurrence and breast cancer-specific mortality mostly in premenopausal women.[34] Large studies in a specific group of patients (regards to age, menopausal status, treatment setting, stage, tumor type, grade and so on) may help us resolve this issue in a systematic manner. While DNA analysis method and endoxifen concentration assay may also be a potential source of variation, most of these methods have been validated and were concordant across various platforms in our review of the clinical studies. A clinical marker for tamoxifen metabolism is the presence of hot flashes. It is considered to be a better marker of tamoxifen treatment outcome. However, only 3 studies evaluated this toxicity based on genotyping, and the findings were not consistent in those studies, further suggesting the need for its evaluation in future studies.[25,31,46]

Since multiple patient- and tumor-specific factors determine the efficacy of tamoxifen in BC patients, large prospective studies are necessary to evaluate the role of *CYP2D6* phenotype, endoxifen concentrations, and concomitant administration with strong CYP2D6 inhibitors on predicting BC outcomes to improve clinical management of BC patients. Because of the highly polymorphic nature of *CYP2D6* that varies among different ethnicities, many variants exist to impact patients' endoxifen concentrations. Therapeutic drug monitoring of endoxifen concentrations may serve well in guiding appropriate tamoxifen dosing or determining whether alternative therapies for BC should be chosen. Two independent studies showed the value of endoxifen concentration monitoring to predict treatment failure.[27,32] Clinical trials are needed to assess the efficacy of higher doses of tamoxifen in patients with low endoxifen steady state concentrations. Currently, there are ongoing or completed studies to evaluate the use of higher tamoxifen dosing in patients who have *CYP2D6*

PM phenotypes and their effects on endoxifen concentrations. The range of endoxifen concentrations observed in the PM category is wide with the mean (8.8 ng ml^{-1}) higher than the concentration (~6 ng ml^{-1}) below which poor clinical outcomes are observed. Furthermore, there is a big discrepancy regarding the effects of *CYP2D6* genotyping on clinical outcomes. Therefore, we believe that using a combination approach including CYP2D6 genotyping with therapeutic drug monitoring of endoxifen concentrations is probably the most ideal strategy to ensure the best clinical outcomes for BC, and deserves further evaluation in future clinical studies.

Our systematic analysis suggests that *CYP2D6* PM phenotype significantly predicts lower endoxifen concentrations in patients receiving a tamoxifen 20 mg once daily regimen. Meanwhile, the overall effects of different *CYP2D6* phenotypes on BC outcomes are less clear. Future well-designed, controlled, larger clinical studies are warranted to determine if *CYP2D6* genotyping and therapeutic drug monitoring of endoxifen concentrations can be used to improve treatment outcomes in BC patients. However, before these trials are initiated, standardization of *CYP2D6* genotype–phenotype classification is needed in order to ensure effective evaluation of associations between CYP2D6 polymorphisms and endoxifen concentrations and BC outcomes. Before such data is available, we recommend that patients who have *CYP2D6* PM phenotypes either be enrolled in appropriate clinical trials evaluating higher than normal dosing of tamoxifen with endoxifen concentration monitoring or be considered for alternative therapies such as aromatase inhibitors. Concomitant administration of strong CYP2D6 inhibitors should be avoided while patients are on tamoxifen. The importance of adequate adherence should also be discussed with all BC patients on tamoxifen regardless of their *CYP2D6* genotype to increase disease-free survival.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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