を併用した群を比較して、response rate で前者が35%に対して後者が45%、time to progression は前者が6.2ヵ月に対して後者が10.6ヵ月と併用療法の有効性が報告された[12]。現状では進行大腸がんの first line として標準的な治療法となっており、さらに adjuvant として、modified FOLFOX6にアバスチンを併用した報告もみられ[13]、さらに FOLFIRI 療法、Capeox療法などの併用療法もすでに広く行われている。また、抗体医薬品を2剤併用する試みも行われている。未治療の転移性大腸がんに対して capecitabine、oxaliplatin そして bevacizumab を投与した群とこれにさらに cetuximab を加えた群を比較した検討である。結果は median progression-free survival で前者が10.7ヵ月に対して後者が9.4ヵ月であり、抗体医薬品を2剤併用した群のほうがむしろ有効性が少ないという結果であった[14]。このことから単純に併用することが有効性の増大には結びつかないことを示している。今後、多くの知見の集積が必要と考えられる。

本邦におけるがん種の多くを占めているのはやはり消化器がんである。一方、このように、現状では、消化器がんに対する標的薬としての抗体医薬は大腸がんに対して大きなインパクトをもって登場している。しかしながら、他の消化器がんに対しては、抗体医薬はまだ発展途上といわざるを得ない。

6. 肝細胞がんに対する抗体医薬開発へ向けて

このような状況から、私たちは肝細胞がんに対する新薬創出のストラテジーを検討してきた。Interferon(IFN)と抗がん剤の投与により、アポトーシスが誘導される可能性についても論じてきた[15,16]。また、このライン上に抗体医薬の開発を試みている。肝細胞がんは現在、残念ながら本邦においてがんの死因の第4位に位置し、また世界的にみてもがんの死因の高位に位置している。特に、肝予備能力も低下した進行した肝細胞がんにおいては、治療の選択肢も少なく、予後の改善が得られていないのが現状である。この進行した肝細胞がんに対する治療薬として、肝予備能力に影響を与えない副作用の少ない抗体医薬はまさにうってつけの治療法と考えられる。

一方、FGFR-1(fibroblast growth factor receptor-1)は、肝細胞がんにおいて、発現されていることが報告され、その進展に関与していることが知られている増殖因子受容体である[17]。われわれのこれまでの FGFR-1に関する分子解析で、*in vitro* および *in vivo* のいずれにおいてもヒト肝がん細胞において、IFN の投与により FGFR-1がさらに過剰発現されることが明らかになった[18]。この FGFR-1は、非がん肝細胞には発現されていないこと、また、FGFR-1を介した刺激は細胞増殖および細胞浸潤、さらに血管新生に関与していることが報告されている。このようなことから、この IFN と抗FGFR-1抗体を併用することで、非がん肝細胞には影響を与えず、すなわち肝予備能力の低下を引き起こさずに、肝がん細胞の増殖抑制、血管新生阻害が可能になることが予想された。このようなことから私たちは、FGFR-1に対する抗体を作製し、IFN との併用によるヒト肝がん細胞に対するその抗がん効果の検討を行ってきたが、*in vitro* および *in vivo* のいずれにおいても、強い抗がん効果が認められている。現在、さらに、この抗体の作用増強をめざした構造改変を行っている。

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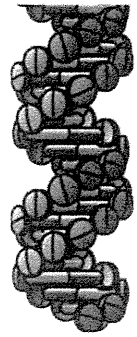
Review

Pharmacogenomics



Pharmacogenomics toward personalized tamoxifen therapy for breast cancer

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Tamoxifen has been used not only for the treatment or prevention of recurrence in patients with estrogen receptor positive breast cancers but also for recurrent breast cancer. Because CYP2D6 is known to be an important enzyme responsible for the generation of the potent tamoxifen metabolite, 'endoxifen', lots of studies reported that genetic variation which reduced its enzyme activity were associated with poor clinical outcome of breast cancer patients treated with tamoxifen. However, there are some discrepant reports questioning the association between *CYP2D6* genotype and clinical outcome after tamoxifen therapy. Dose-adjustment study of tamoxifen based on *CYP2D6* genotypes provides the evidence that dose adjustment is useful for the patients carrying reduced or null allele of *CYP2D6* to maintain the effective endoxifen level. This review describes critical issues in pharmacogenomic studies as well as summarizes the results of the association of *CYP2D6* genotype with tamoxifen efficacy.

Keywords: ABCC2 • C10orf11 • CYP2D6 • dose adjustment • endoxifen • pharmacogenomics • tamoxifen

Tamoxifen has been widely used for the treatment of patients with estrogen receptor (ER) positive breast cancer mainly in adjuvant setting. The clinical benefit of this drug for the treatment of ER-positive early breast cancer is evident because recurrence and mortality rates are reduced by this drug [1]. Tamoxifen therapy for 5 years was reported to improve the risk of its relapse at least for 15 years, particularly ER-positive invasive tumors in premenopausal women [1]. However, in the result of Adjuvant Tamoxifen Longer Against the Shorter (ATLAS), the risk of recurrence during years 5–14 was greater than 20% in the patients treated with adjuvant tamoxifen therapy [2]. Although results of many studies are accumulated, the mechanisms underlying the resistance to this drug in a subset of the patients are not fully identified. Tamoxifen is, in a sense, a prodrug that requires metabolic activation to induce pharmacological activity. 4-hydroxytamoxifen and endoxifen (4-hydroxy-N-desmethyltamoxifen), which are representative metabolites of tamoxifen, are reported to be active therapeutic moieties [3]. These two metabolites have 100-fold greater affinity to ER and 30- to 100-fold greater potency in inhibiting estrogen-dependent cell growth compared with tamoxifen [3–5]. The differences in the formation of these active metabolites could affect the interindividual variability in the response to tamoxifen. CYP2D6 is well known to be one of the important enzymes for the generation of 4-hydroxytamoxifen and endoxifen. Lots of genetic polymorphisms of *CYP2D6* including alleles that alter the function and/or amount of the gene product have been reported [6]. CYP2D6 is usually classified into three groups according to the phenotypes: poor metabolizers (PMs), intermediate metabolizers (IMs) and extensive metabolizers (EMs). Patients with two null alleles are classified as PMs of drugs that are metabolized by CYP2D6 [7–9]. The major null alleles that cause the PMs in Cau-

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casians are *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*5* and *CYP2D6*6*, and account for nearly 95% of the PMs [10]. Patients classified into PMs have been reported to have lower plasma levels of endoxifen and poorer clinical outcomes when treated with tamoxifen [11]. On the other hand, although the frequency of PMs in Asians is much lower (<1%), the *CYP2D6*10* allele, which causes amino acid substitutions and reduce the enzymatic activity, has been observed in 40–50% of Asians [12,13]. The influence of *CYP2D6*10* to clinical outcome after tamoxifen therapy in adjuvant setting has been investigated [12]. Several research groups reported that the association of genetic polymorphisms of *CYP2D6* with clinical efficacy of tamoxifen [14–17], indicating the association with the plasma concentrations of the active metabolites [14,18]. Genotype-guided dose-adjustment studies for tamoxifen, which showed steady-state plasma concentrations of tamoxifen and its metabolites, have been reported to investigate the optimal dosage for each patient with breast cancer [9,19,20]. However, some studies showed negative results for the association between *CYP2D6* genotype and response to tamoxifen [21–23]. There may be several reasons for these discrepant results showing the positive and negative associations. The mechanism which causes interindividual differences in responsiveness to tamoxifen is not yet fully clarified even if the effects of genetic variation of *CYP2D6* were considered. This review comments on several factors that may have influenced the conclusions of CYP2D6–tamoxifen association studies. This process may inform some general interpretive rules around the literature of the association between *CYP2D6* genotype and tamoxifen responsiveness [24].

Metabolic pathway of tamoxifen

Tamoxifen has been known to be metabolized in liver and gut wall in humans to primary and secondary metabolites that exhibit a range of pharmacologic activity [25,26]. Therefore, the differences in systemic exposure of the active metabolites should contribute to the variable response of tamoxifen observed in patients with breast cancer [27]. Tamoxifen can be considered a prodrug because the parent drug itself does not have strong affinity for the ER but undergoes extensive biotransformation into active and inactive metabolites. CYP450 enzymes such as CYP2C9, CYP2C19, CYP2D6, CYP2B6 and CYP3A are active in this metabolic process (Figure 1). 4-hydroxytamoxifen (4-OH tamoxifen) had been reported to play an important role as active metabolite of tamoxifen. It has high affinity for ERs and 30- to 100-fold greater potency than tamoxifen in inhibiting estrogen-dependent breast cancer cell proliferation [26,28]. However, another metabolite of tamoxifen, endoxifen (4-hydroxy-N-desmethyltamoxifen), was identified (Figure 1) [29]. In the course of the metabolism of tamoxifen to 4-OH tamoxifen by multiple enzymes, endoxifen is formed preponderantly by the CYP2D6-mediated oxidation of N-desmethyl tamoxifen, which is N-desmethylated by the CYP3A enzyme (Figure 1) [30]. Endoxifen was reported in the 1980s in humans [29], however, its role had remained unknown. Endoxifen, which has high affinity for ERs and greater potency than tamoxifen, reaches greater than sixfold higher plasma concentrations than 4-OH tamoxifen in patients taking tamoxifen [26]. The hydroxylated metabolites undergo conjugation by sulfotransferases (SULTs) and/or uridine diphosphate glucuronosyltransferases (UGTs) (Figure 1) [31–35].

Association of *CYP2D6* genotype with its enzyme activity

The *CYP2D6* gene, which is located on chromosome 22, has many genetic variations with greater than 80 major alleles currently known [36]. A subset of the variations affect the gene product and result in wide interindividual and ethnic differences in CYP2D6 activity (Table 1) [6,36]. CYP2D6 plays a key role in the biotransformation of many drugs including selective serotonin reuptake inhibitors, antidepressants, antiarrythmics and neuroleptics [37]. Some of these alleles are associated with decreased enzyme activity (e.g., **9*, **10*, **17*, **41*) or null enzyme activity (e.g., **3*, **4*, **5*, **6*) as shown in Table 1. Large interindividual and ethnic variability in the metabolism of drugs by CYP2D6 suggests the genetic polymorphisms affecting the enzyme activity and gene expression [38]. The concentrations of endoxifen could vary significantly in patients treated with tamoxifen due to *CYP2D6* genetic variation [14,27]. An *in vitro* study, in which breast cancer cells are exposed to clinically equivalent concentrations of tamoxifen and its metabolites, reported that ER-α degradation and transcription caused by endoxifen was concentration dependent, with minimal effect at lower endoxifen concentrations observed in PMs, but significantly greater effects occurring at concentrations observed in IMs and EMs [22,39]. These data are considered to support the theory that endoxifen is one of the most important tamoxifen metabolites [18]. Moreover, a pharmacogenomic study reported that endoxifen concentration varied according to the number of functional *CYP2D6* alleles [40].

Effect of CYP2D6 inhibitors on the response to tamoxifen

Medical drugs, which are metabolized by CYP2D6, also affect plasma endoxifen level. Concomitant CYP2D6 inhibitor use during tamoxifen therapy has

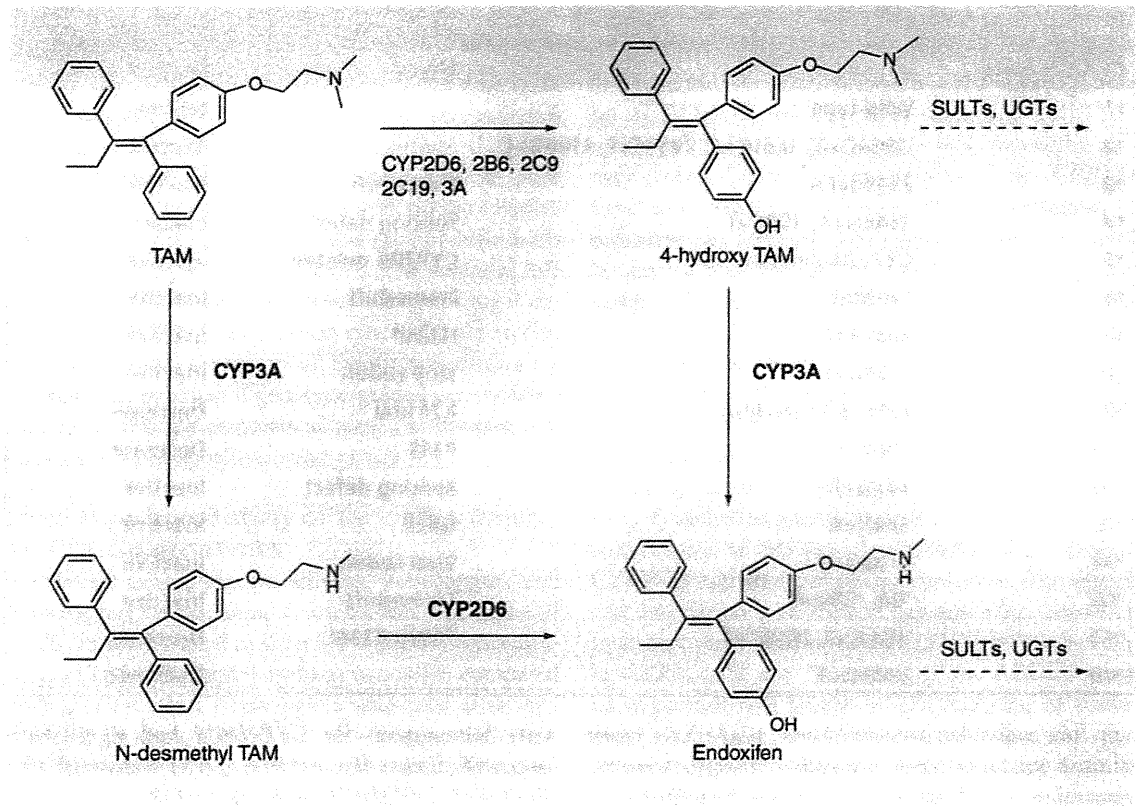

Figure 1. Metabolism of tamoxifen by the CYP450 system. The hydroxylated metabolites undergo conjugation by SULTs and UGTs.
SULT: Sulfotransferase; TAM: Tamoxifen; UGT: Uridine diphosphate glucuronosyltransferase.

been seen in about 30% of patients with breast cancer [27]. Many studies have investigated CYP2D6 inhibitory potential of medications. Generally, CYP2D6 inhibitors were classified into weak or moderate (sertraline, citalopram, fluvoxamine, etc), and strong inhibitors (paroxetine, fluoxetine, bupropion, duloxetine, etc) [39]. According to the results reported by Flockhart and colleagues, the SSRI and the selective norepinephrine reuptake inhibitors, especially strong inhibitors (fluoxetine and paroxetine), would be critical in tamoxifen pharmacogenetics because they are used in patients with breast cancer to treat hot flashes and depression [41–44]. However, the drugs classified as weak inhibitors, including sertraline and citalopram, are considered to have little or no impacts on the tamoxifen treatment [45]. Several groups reported the effects of concurrent use of CYP2D6 inhibitors with tamoxifen on the risk of the recurrence [46–50]. Fifteen drugs inhibiting CYP2D6 were investigated by Ahern *et al.*, and a null association on breast cancer recurrence was observed in the patients treated with tamoxifen [46]. However, the patients co-administrated with paroxetine were likely to show higher odds ratio [43]. Kelly *et al.* reported that absolute increases of the period for concomitant use of paroxetine with tamoxifen were significantly associated with increases in the risk of death from breast cancer, however, the other SSRIs did not [47]. Goetz *et al.* reported the significant effects of both of *CYP2D6* genotype and CYP2D6 inhibitors; however, questions remain in the contribution of CYP2D6 inhibitors to the results [15]. Further investigation considering these issues are required, however, these lines of evidence suggest that concurrent use of strong CYP2D6 inhibitors, especially paroxetine and possibly the others, should be avoided in the breast cancer patients treated with tamoxifen [51].

CYP2D6 genotype & clinical outcome after tamoxifen therapy

Endoxifen has antiestrogen effect in breast cancer cells that functions by targeting ER-α for degradation by the proteasome, blocking ER-α transcriptional activity and reducing estrogen-induced breast cancer cell growth [39]. Recently, an explosion of interest has been seen in the effect of *CYP2D6* genotype on clinical outcomes for breast cancer patients treated with tamoxifen [52]. There has been hypothesis that women with a reduced CYP2D6 enzyme activity, and thus presum-

Table 1. Genetic variation of *CYP2D6* and its enzyme activity.

CYP2D6 allele	Variant	Effect	Enzyme activity
**1*	Wild-type		Normal
**2*	-1584C>G, 1661G>C, 2850C>T, 4180G>C		Normal
**3*	2549del A	Frameshift	Inactive
**4*	1846G>A; 100C>T	Splicing defect	Inactive
**5*	CYP2D6 deletion	CYP2D6 deleted	Inactive
**6*	1707delT	Frameshift	Inactive
**7*	2935A>C	H324P	Inactive
**8*	1758G>T	Stop codon	Inactive
**9*	2613_2615 delAGA	K281del	Decrease
**10*	100C>T	P34S	Decrease
**11*	883G>C	Splicing defect	Inactive
**12*	124G>A	G42R	Inactive
**14*	1758G>A	Stop codon	Inactive
**15*	138_139InsT	Frameshift	Inactive
**17*	1023C>T, 2850C>T	T107I; R296C	Decrease
**41*	2988G>A		Decrease

ably low endoxifen concentrations, might have worse clinical outcomes after tamoxifen therapy; however, generation of definitive proof of this hypothesis has been controversial [52].

Many clinical trials as to this association have been reported until today. One of the first studies was reported by researchers in Mayo Clinic who determined *CYP2D6* genotype by extraction of DNA from postmenopausal women treated with 5-year-tamoxifen [15]. They reported that carriers of a *CYP2D6*4* (null of enzymatic activity) had a significantly shorter time-to-recurrence and relapse-free survival (HR: 1.74; p = 0.017) than those in EMs [15]. Kiyotani *et al.* reported that *CYP2D6* variants which reduce or lack enzymatic activity were significantly associated with shorter recurrence-free survival (RFS) in 282 Japanese patients receiving adjuvant tamoxifen monotherapy (HR: 9.52; 95% CI: 2.79–32.45; p = 0.000036 in patients with two variant alleles vs patients without variant alleles) [14]. However, two retrospective studies reported that no association was found between *CYP2D6* genotype and the clinical outcomes in 2005 [22,53]. Nowell *et al.* carried out a retrospective study and reported a trend toward longer overall survival in a cohort of adjuvant tamoxifen-treated breast cancer patients with *CYP2D6*4* allele [22]. A Swedish study also reported the improved outcomes in patients with at least one *CYP2D6*4* allele who were treated with tamoxifen for 2 years after surgical operation [53]. The other larger cohort by the same group also suggested that women with ER-positive breast cancer who were homozygous for *CYP2D6*4* had significantly improved disease-free survival (DFS) compared with those with *CYP2D6*1* (wild-type) [23,52].

In addition to the above trial, many clinical studies reported the relationship between *CYP2D6* genotype and clinical outcome of patients treated with tamoxifen in adjuvant or metastatic setting [16,18,54–68] (Table 2). In 2009, Schroth *et al.* subsequently published a retrospective analysis of 1325 German and North American patients with early-stage breast cancer treated with tamoxifen in adjuvant setting [16]. With a median follow-up of 6.3 years, the authors observed that women with reduced CYP2D6 activity (heterozygous for either a reduced activity or null activity allele: IMs or PMs) had a significantly poor clinical outcome (HR: 1.4; 95% CI: 1.04–1.9) compared with EMs [16]. Although PMs were at increased risk of recurrence compared with their EM counterparts with a time to recurrence HR of 1.9 (95% CI: 1.1–3.28), a significant difference in overall survival was not observed [16]. In contrast, two large studies, the Arimidex, Tamoxifen, Alone or in Combination (ATAC) and Breast International Group (BIG) 1–98 trials, for the relationship between *CYP2D6* genotype and clinical outcome after tamoxifen therapy recently reported that the relationship has not been confirmed [21,69]. However, all of the above studies including ATAC and BIG 1–98 were retrospective studies and lack for the uniformity in genotyping method, coverage of genotyped alleles, DNA source and dose of tamoxifen [70,71]. Some authors do not recommend routine use of the *CYP2D6*

test until robust confirmatory data are available from adequately powered prospective trials [72,73].

Recently, as a result of meta-analysis on data from 4973 tamoxifen-treated patients, the International Tamoxifen Pharmacogenomics Consortium (12 globally distributed sites) reported that CYP2D6 poor metabolizer status was associated with poorer invasive DFS using strict inclusion criteria (IDFS: HR: 1.25; 95% CI: 1.06, 1.47; p = 0.009) [17]. The potential role of *CYP2D6* genotype assessment in determining if the patients with ER-positive breast cancer should receive tamoxifen is still controversial. Prospective studies are necessary to establish if genotype-guided personalized tamoxifen therapy improves clinical outcomes of the patients with ER-positive breast cancer [17].

Dose-adjustment study of tamoxifen based on *CYP2D6* genotypes

The breast cancer patients who are heterozygous and homozygous for decreased function and null alleles of *CYP2D6* are reported to show lower plasma concentrations of endoxifen and 4-hydroxytamoxifen compared with patients with homozygous wild-type allele [27], resulting in worse clinical outcome in tamoxifen therapy. Kiyotani *et al.* reported tamoxifen dose adjustment study using 98 Japanese breast cancer patients, who had been taking 20 mg of tamoxifen daily as adjuvant setting [19]. In their study, dosages of tamoxifen were increased to 30 and 40 mg/day for the patients who have one or no normal allele of *CYP2D6*, respectively. In the patients with *CYP2D6*1/*10* and *CYP2D6*10/*10*, the plasma endoxifen levels after dose increase were 1.4- and 1.7-fold higher, respectively, than those before the increase ($p < 0.001$) [19]. These plasma concentrations of endoxifen achieved similar level of those in the *CYP2D6* wild-type patients receiving 20 mg/day of tamoxifen. In addition, they showed that the incidence of adverse events was not significantly different between before and after dose adjustment, and concluded that their study provided the evidence that dose adjustment could be useful for the patients carrying *CYP2D6*10* allele to maintain the effective endoxifen level. Similar genotype-guided tamoxifen dosing study was reported [9,20]. Irvin *et al.* also showed the similar results, and the feasibility of genotype-driven tamoxifen dosing and demonstrates that doubling the tamoxifen dose can increase endoxifen concentrations in IM and PM patients [9].

Possible genetic markers for clinical response to tamoxifen

As shown in Figure 1, UGTs, SULTs and the other CYPs are involved in the metabolism of tamoxifen. Some reports suggest that genetic variations in these genes may affect the efficacy or toxicity of tamoxifen therapy [14,22,27,76–79]. Several genetic polymorphisms are reported in *SULT1A1*, and some investigations on *SULT1A1*2*, which causes decreased SULT1A1 activity, failed to find association with tamoxifen efficacy [23,80]. Genetic polymorphisms in the *CYP3A4* have been reported, however, their contribution to influence the tamoxifen metabolism might be small because of their low allelic frequencies. On the other hand, *CYP3A5*3* allele is known to influence to CYP3A5 expression level [81]. Several studies investigated the association of *CYP3A5*3* with tamoxifen metabolism or clinical outcome of tamoxifen therapy, however, none of them report their significant association [27,74–76,81–83]. *CYP2C19*2* and *CYP2C19*3* are known to be null allele, and *CYP2C19*17*, which is recently identified genetic variation and located in promoter region of this gene, is associated with increased CYP2C19 activity (UM phenotype) [77,78]. The significant association with clinical outcome after tamoxifen treatment was found in *CYP2C19*17* carriers, but not in *CYP2C19*2* nor **3* carriers [77,78]. ABCC2 plays an important role in the biliary excretion of conjugated drugs and xenobiotics [84,85]. Tamoxifen and its metabolites are excreted into the biliary tract in liver as glucuronides or sulfates [86]. In a recent study, an intronic SNP in *ABCC2* was found to be significantly associated with the clinical outcome of breast cancer patients treated with tamoxifen, however, this SNP was not associated with plasma concentration of endoxifen or other metabolites [14]. This suggests that the contribution of ABCC2 to biliary excretion of tamoxifen and its metabolites might be limited. A genome-wide association study for clinical outcome of the breast cancer patients treated with tamoxifen was reported [79]. In this study, 240 patients were analyzed by genome-wide genotyping, and 105 and 117 cases were used for replication studies as independent cohorts, respectively. Out of 15 SNPs which showed significant associations with recurrence-free survival in genome-wide association study stage, rs10509373 in *C10orf11* gene on 10q22 was significantly associated with tamoxifen efficacy in the two independent replication stages [79]. Although further validation studies and functional analysis would be required to verify their results, *C10orf11* could be a promising genetic marker to predict the clinical outcomes of patients receiving tamoxifen therapy [79].

Conclusion

There have been several reports on the association between *CYP2D6* genotype and clinical outcome or tamoxifen metabolism in breast cancer patients treated with tamoxifen. The results of the association studies

Table 2. Studies evaluating association of *CYP2D6* genotype with response to tamoxifen therapy.

Study findings	Studies	n	Ratio of monotherapy (%)	Tamoxifen dose and duration	Outcome[†]	HR (95% CI)	p-value	Ref.
Positive	Goetz *et al.*	190	–	20 mg/day for 5 years	DFS	2.44 (1.22–4.90)	0.012	[74]
	Goetz *et al.*	180	100	20 mg/day for 5 years	RFS	2.69 (1.34–5.37)	0.005	[15]
	Schroth *et al.*	206	100	–	RFS	2.24 (1.16–4.33)	0.02	[75]
	Newman *et al.*	115	63.5	20 mg/day, median duration >4 years	RFS	1.9 (0.8–4.8)	0.19	[58]
	Kiyotani *et al.*	58	100	20 mg/day for 5 years	RFS	8.67 (1.06–71.09)	0.044	[12]
	Xu *et al.*	152	100	–	DFS	4.7 (1.1–20.0)	0.04	[54]
	Schroth *et al.*	1325	100	For 5 years	RFS	2.12 (1.28–3.50)	0.003	[16]
	Kiyotani *et al.*	282	100	20 mg/day for 5 years	RFS	9.52 (2.79–32.45)	0.0032	[14]
	Ramon *et al.*	91	39.8	–	DFS		0.016	[55]
	Park *et al.*	110	21.80	20 mg/day, median duration 3.9 years	RFS	5.59 (0.93–33.5)	0.05	[67]
	Thompson *et al.*	542	100	20 mg/day for 5 years	RFS	1.52 (0.98–2.36)	0.06	[68]
	Teh *et al.*	95	–	20 mg/day	RFS	13.14 (1.54–109.9)	0.004	[59]
	Sirachainan *et al.*	39	100	–	DFS		0.036	[60]
	Damodaran *et al.*	132	6.80	For 5 years	RFS	7.15 (1.77–28.89)	0.006	[61]
	Goetz *et al.*	453	100	20 mg/day for 5 years	Disease event	2.45 (1.05–5.73)	0.04	[57]
	Province *et al.*	4973 (1996)	100	20 mg/day for 5 years	IDFS	1.25 (1.06–1.47)	0.009	[17]
Negative	Wegman *et al.*	76	50	40 mg/day for 2 years	RFS	<1.0[‡]	–	[53]
	Nowell *et al.*	160	14.2	Not reported	DFS	0.67 (0.33–1.35)	0.19	[22]
	Wegman *et al.*	103	–	40 mg/day for 2 years	RFS	0.87 (0.38–1.97)	0.74	[23]
		111	–	40 mg/day for 5 years	RFS	0.33 (0.08–1.43)	0.14	
	Okishiro *et al.*	173	42.2	20 mg/day, median 52 months	RFS	0.94 (0.34–2.60)	0.95	[62]
	Stingl *et al.*	493	58	20 mg/day	TTP	–	0.10	[63]
	Kiyotani *et al.*	167	0	20 mg/day for 5 years	RFS	0.64 (0.20–1.99)	0.44	[64]
	Abraham *et al.*	3155	48.4	20 mg/day	RFS	1.57 (0.64–3.84)	0.32	[56]
	Lash *et al.*	340	–	–	Disease event	1.3 (0.60–2.9)	0.88	[65]
	Park *et al.*	130	18.2	–	RFS	1.34 (0.42–4.28)	0.63	[66]
	Rae *et al.*	588	95.7	20 mg/day for 5 years	RFS	1.22 (0.76–1.96)	0.44	[69]
	Regan *et al.*	973	100	20 mg/day for 5 years	RFS	0.58 (0.28–1.21)	0.35	[21]

All reports were retrospective studies.
[†]RFS was defined as time from surgery or randomization to diagnosis of the recurrence of breast cancer (locoregional, distant metastasis and contralateral breast events). DFS was defined as time from surgery or randomization to diagnosis of the recurrence of breast cancer or death. IDFS specifically excludes all *in situ* cancer events (ipsilateral or contralateral DCIS, ipsilateral or contralateral LCIS and all *in situ* cancers of nonbreast sites).
[‡]Not calculated HR according to *CYP2D6* genotypes.
DFS: Disease-free survival; HR: Hazard ratio; IDFS: Invasive DFS; RFS: Recurrence-free survival.

of tamoxifen metabolism with *CYP2D6* genotype are consistent in most of the studies, however, the results of the association studies of tamoxifen efficacy with *CYP2D6* genotype are still controversial. Although there might be several reasons for these controversial results, well-designed prospective studies will clarify if *CYP2D6* genotype test could improve the outcomes of women with ER-positive breast cancer. Moreover, the combined genetic test of *CYP2D6* with a few predictive genetic markers may provide new insights into personalized selection of hormonal therapy for the patients with breast cancer. The potent CYP2D6 inhibitors including paroxetine should be avoided in the breast cancer patients receiving tamoxifen as alternative treatment should be available in most cases.

Future perspective

The dose-adjustment studies based on the *CYP2D6* genotypes showed that the increase of tamoxifen dose was able to increase the plasma endoxifen level, and expected to improve the prognosis of the tamoxifen-treated patients with reduced *CYP2D6* genotype [9,19]. A large-scale prospective study will clarify whether the dose-adjustment strategy could improve tamoxifen therapy in breast cancer patients. Although there are some discrepant reports questioning the association between *CYP2D6* genotype and clinical outcome after tamoxifen therapy, one of the largest meta-analysis performed by International Tamoxifen Pharmacogenomics Consortium reported that *CYP2D6* could be a strong predictor of invasive DFS using strict inclusion criteria (postmenopausal women with ER-positive breast cancer receiving 20 mg/day tamoxifen for 5 years). In either case, prospective studies are essential to finally conclude if genotype-guided selection of tamoxifen therapy improves clinical outcomes of women with ER-positive breast cancer. If the results will show the positive association of *CYP2D6* genotype with clinical outcome of tamoxifen-treated patients, US FDA may approve and recommend routine use of the *CYP2D6* genotype test for personalized tamoxifen therapy in adjuvant or metastatic breast cancer setting.

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Executive summary

- Tamoxifen treatment reduced the risk of breast cancer relapse for at least 15 years, particularly estrogen receptor positive invasive tumors in premenopausal women.
- CYP2D6 is known to be a key enzyme to generate one of the potent tamoxifen metabolites, endoxifen.
- Although there are some discrepant reports questioning the association between *CYP2D6* genotype and clinical outcome after tamoxifen therapy, the highest level of evidence to test the CYP2D6–tamoxifen hypothesis will come from larger scale prospective clinical trials.
- Combined analysis of newly identified genetic marker(s) with previously identified ones, *CYP2D6*, *ABCC2* and so on, might be useful to predict the clinical outcome of patients receiving tamoxifen therapy.

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