Review

Pharmacogenomics of Tamoxifen: Roles of Drug Metabolizing Enzymes and Transporters

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Summary: Tamoxifen has been widely used for the prevention of recurrence in patients with hormone receptor-positive breast cancer. Tamoxifen requires metabolic activation by cytochrome P450 (CYP) enzymes for formation of active metabolites, 4-hydroxytamoxifen and endoxifen, which have 30- to 100-fold greater affinity to the estrogen receptor and the potency to suppress estrogen-dependent breast cancer cell proliferation. CYP2D6 is a key enzyme in this metabolic activation and it has been suggested that the genetic polymorphisms of CYP2D6 influence the plasma concentrations of active tamoxifen metabolites and clinical outcomes for breast cancer patients treated with tamoxifen. The genetic polymorphisms in the other drug-metabolizing enzymes, including other CYP isoforms, sulfotransferases and UDP-glucuronosyltransferases might contribute to individual differences in the tamoxifen metabolism and clinical outcome of tamoxifen therapy although their contributions would be small. Recently, involvement of a drug transporter in the disposition of active tamoxifen metabolites was identified. The genetic polymorphisms of transporter genes have the potential to improve the prediction of clinical outcome for the treatment of hormone receptor-positive breast cancer. This review summarizes current knowledge on the roles of polymorphisms in the drug-metabolizing enzymes and transporters in tamoxifen pharmacogenomics.

Keywords: P450 2D6; MRP2; MDR1; UGT; SULT; single nucleotide polymorphism; endoxifen; estrogen receptor

Introduction

Tamoxifen, a selective estrogen receptor (ER) modulator, has been widely used for the prevention and treatment of hormone receptor-positive breast cancers in more than 120 countries throughout the world. Since most breast cancers are hormone receptor-positive, thousands of breast cancer patients worldwide initiate endocrine treatment each year. Based on the results of the Early Breast Cancer Trials’ Collaborative Group, the standard recommendation has been 5 years of therapy with tamoxifen.1) In pre- and postmenopausal patients with primary breast cancer, adjuvant tamoxifen significantly decreased recurrence and breast cancer mortality for 15 years after primary diagnosis.1) However, 30–50% of patients with adjuvant tamoxifen therapy experience relapse and subsequently die of the disease1,2) indicating individual differences in responsiveness to tamoxifen.

Tamoxifen is extensively metabolized to more active or inactive metabolites by phase I and phase II enzymes, including cytochrome P450s (CYPs), sulfotransferases (SULTs) and UDP-glucuronosyltransferases (UGTs). Recent data support that the polymorphisms in these drug-metabolizing enzymes contribute to individual differences in plasma concentrations of active tamoxifen metabolites and tamoxifen clinical outcome. Among them, CYP2D6 is most extensively investigated. It was recently reported that drug transporters are involved in the transport of active tamoxifen metabolites and it is suggested that the polymorphisms of
transporter genes are likely to be involved in variable clinical outcome observed in patients treated with tamoxifen. This review summarizes current data on the relationships of genetic polymorphisms of the tamoxifen-metabolizing enzymes and transporters to individual differences in tamoxifen disposition and clinical outcomes of breast cancer patients with tamoxifen treatment.

**Tamoxifen Metabolism**

Tamoxifen is extensively metabolized by phase I and phase II enzymes in the human liver (Fig. 1). The parent drug itself has weak affinity to the ER, only 1.8% of the affinity of 17β-estradiol.3 The major metabolite N-desmethyltamoxifen is formed by N-demethylation, which is catalyzed mainly by CYP3A4 and CYP3A5, with minor contribution by CYP2D6, CYP1A2, CYP2C9 and CYP2C19.4-6 The steady state plasma concentration of N-desmethyltamoxifen after administration of 20 mg/day tamoxifen is approximately twice as high as that of tamoxifen.7,8 N-Desmethyltamoxifen shows weak affinity to the ER, similar to that of tamoxifen.3 4-Hydroxytamoxifen, which is formed by 4-hydroxylation of tamoxifen, had been considered to play an important role as an active metabolite because it has 100-fold higher affinity to the ER and 30- to 100-fold greater potency than tamoxifen in suppressing estrogen-dependent breast cancer cell proliferation.3,9-11 This conversion is catalyzed by CYP2D6, CYP2B6, CYP2C9, CYP2C19 and CYP3A4.6,12-14 A different metabolite, 4-hydroxy-N-desmethyltamoxifen (endoxifen), was identified in the 1980s in humans but its role had remained unknown. Recent reports have clarified that endoxifen has a potency equivalent to 4-hydroxytamoxifen,9,15,16 and plasma endoxifen levels exceed plasma concentration levels of 4-hydroxytamoxifen by several folds, suggesting endoxifen to be a principal active metabolite.8,10 Although the metabolism of tamoxifen to 4-hydroxytamoxifen is catalyzed by multiple isoforms, endoxifen is formed predominantly by the CYP2D6-mediated 4-hydroxylation of N-desmethyltamoxifen.17 In addition, N-desmethyltamoxifen can also be demethylated by CYP3A4 to form N,N-didesmethyltamoxifen. Further hydroxylation also takes place at the 4’ position, leading to 4’-hydroxytamoxifen, which is mainly mediated by CYP2B6 and CYP2D6, and to 4’-hydroxy-N-desmethyltamoxifen.6 Another hydroxylated metabolite, α-hydroxytamoxifen, is produced mainly by CYP3A4.4,5 However, except for endoxifen and 4-hydroxytamoxifen, no other highly active metabolites have been described so far.18

Tamoxifen and these metabolites are further metabolized by phase II enzymes, such as SULTs and UGTs. SULT1A1 is considered to be the primary SULT responsible for the sulfation of 4-hydroxytamoxifen and endoxifen.19,20 UGT1A8, UGT1A10, UGT2B7, UGT2B15 and UGT1A4 are involved in the O-glucuronidation of 4-hydroxytamoxifen and endoxifen.21-23 Tamoxifen and 4-hydroxytamoxifen are glucuronidated by UGT1A4 to the corresponding
The genetic variations of these drug-metabolizing enzymes have the potential to affect tamoxifen metabolism.

**Genetic Polymorphisms of CYP2D6**

CYP2D6 is one of the most important CYP isoforms due to its central role in the metabolism of a number of clinically important drugs, including β-blockers, antihypertensives, antipsychotics, antidepressants, opioids and others. The CYP2D6 gene is located on chromosome 22q13.1, containing two neighboring pseudogenes, CYP2D7 and CYP2D8. This locus is extremely polymorphic with over 80 allelic variants, as presented at the home page of the human CYP allele nomenclature committee (http://www.cypalleles.ki.se/cyp2d6.htm), which should be one of the causes of wide inter-individual and ethnic differences in CYP2D6 activity in vivo. Commonly, four CYP2D6 phenotypes are observed on the basis of their metabolic capacities: extensive metabolizer (EM), poor metabolizer (PM), intermediate metabolizer (IM) and ultra-rapid metabolizer (UM). It has been reported that the PM phenotype, which is caused by the carrying of two null alleles, reveals itself in 5–10% of Caucasians. CYP2D6*3, CYP2D6*4, CYP2D6*5 and CYP2D6*6 are major null alleles that cause the PM phenotype and account for nearly 95% of the PMs in Caucasians (Table 1). In contrast, less than 1% of Asians show the PM phenotype, and most Asians are categorized as IMs due to frequent carries of CYP2D6*10 alleles. The CYP2D6*14, CYP2D6*18, CYP2D6*21, CYP2D6*44 alleles were found as null alleles in Asian populations, although their frequencies are very low. The frequencies of UMs, who are carriers of duplicated/multiplied CYP2D6 gene, are 10–15% in Caucasian, whereas UMs are uncommon in Asians.

The CYP2D6 genotype-phenotype relationship was well investigated. In the patients who are PMs or IMs, tamoxifen is not metabolized effectively to its active metabolites and therefore would provide little anti-estrogenic effect. With respect to UMs, it is important to note that such patients may be more susceptible to hot flashes during tamoxifen therapy.

**CYP2D6 genotype and clinical outcome of tamoxifen therapy:** In recent years, we have seen an explosion of interest in the clinical relevance of CYP2D6 genotype on outcomes for breast cancer patients treated with tamoxifen. It has been hypothesized that patients with a lower CYP2D6 activity due to genetic variations may show lower endoxifen concentration in plasma, and thus might have poorer clinical outcome.

Prospective cohort studies of adjuvant tamoxifen treatment have revealed wide inter-individual variation in the steady-state plasma concentrations of active metabolites, endoxifen and 4-hydroxytamoxifen, during tamoxifen treatment in women carrying CYP2D6 gene variants. The patients homozygous for null alleles (categorized as PM) show four-fold lower concentration of endoxifen in plasma than those carrying two normal alleles (categorized as EM). The low function alleles, including CYP2D6*10 and CYP2D6*41, were also reported to cause insufficient formation of endoxifen from the data that the patients carrying two low-function alleles (categorized as IM) had two-fold lower plasma endoxifen concentration.

Moreover, convincing evidence has shown that selective serotonin reuptake inhibitors such as paroxetine and fluoxetine, which are known to be strong CYP2D6 inhibitors, reduced plasma endoxifen concentration.

As shown in Table 2, a number of the clinical trials have reported the association between the CYP2D6 genotype and clinical outcome of breast cancer patients having tamoxifen therapy. One of the first studies reported by Goetz et al. in 2005 demonstrated that homozygous carriers of a CYP2D6*4 allele had a shorter relapse-free survival (RFS) and disease-free survival (DFS) compared with the patients heterozygous or homozygous for the wild-type allele (hazard ratio (HR), 1.85; p = 0.18 for RFS; HR, 1.86; p = 0.089 for DFS). As a follow-up study, they reported that the patients classified as PMs and IMs had a significantly shorter time to recurrence (HR = 1.91; p = 0.034) and worse RFS (HR = 1.74; p = 0.017) relative to EMs. Schroth et al. reported significantly shorter RFS (HR, 2.24; p = 0.02) among patients carrying the CYP2D6*4, CYP2D6*5, CYP2D6*10 and CYP2D6*41 alleles, compared with patients with two functional alleles in a study of a German population.
Table 2. Summary of studies evaluating association of CYP2D6 genotype with response to adjuvant tamoxifen therapy

<table>
<thead>
<tr>
<th>Studies</th>
<th>Number of patients</th>
<th>Tamoxifen therapy</th>
<th>% of monotherapy</th>
<th>Tamoxifen dose</th>
<th>Outcome</th>
<th>Univariate</th>
<th>Hazard ratio (95% CI)</th>
<th>p value</th>
<th>Multivariate</th>
<th>Hazard ratio (95% CI)</th>
<th>p value</th>
<th>Genotype groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goetz et al., 2005</td>
<td>190</td>
<td>Monotherapy</td>
<td>100%</td>
<td>20 mg/day for 5 years</td>
<td>DFS</td>
<td>2.44 (1.22–4.90)</td>
<td>0.012</td>
<td>1.86 (0.91–3.82)</td>
<td>0.089</td>
<td></td>
<td></td>
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<tr>
<td>Wegman et al., 2005</td>
<td>76</td>
<td>+Chemotherapy or radiation</td>
<td>not reported</td>
<td>40 mg/day for 2 years</td>
<td>RFS</td>
<td>not reported</td>
<td>&lt;1.0</td>
<td>not reported</td>
<td>wt/wt vs wt/*4/*4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nowell et al., 2005</td>
<td>160</td>
<td>+Chemotherapy or radiation</td>
<td>14.2%</td>
<td>not reported</td>
<td>DFS</td>
<td>not reported</td>
<td>0.67 (0.33–1.35)</td>
<td>0.19</td>
<td>wt/wt vs wt/*4/*4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goetz et al., 2007</td>
<td>180</td>
<td>Monotherapy</td>
<td>100%</td>
<td>20 mg/day for 5 years</td>
<td>RFS</td>
<td>3.20 (1.37–7.55)</td>
<td>0.007</td>
<td>not reported</td>
<td>wt/wt vs PM</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Wegman et al., 2007</td>
<td>103</td>
<td>not reported</td>
<td>not reported</td>
<td>40 mg/day for 2 years</td>
<td>RFS</td>
<td>not reported</td>
<td>0.87 (0.38–1.97)</td>
<td>0.74</td>
<td>wt/wt vs wt/*4/*4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schroth et al., 2007</td>
<td>206</td>
<td>Monotherapy</td>
<td>100%</td>
<td>not reported</td>
<td>RFS</td>
<td>not reported</td>
<td>2.24 (1.16–4.33)</td>
<td>0.02</td>
<td>EM vs decreased</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newman et al., 2008</td>
<td>115</td>
<td>Monotherapy or +chemotherapy and/or radiation</td>
<td>63.5%</td>
<td>20 mg/day, median duration &gt;4 years</td>
<td>RFS</td>
<td>not reported</td>
<td>1.9 (0.8–4.8)</td>
<td>0.19</td>
<td>wt/wt vs wt/*4/*4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kiyotani et al., 2008</td>
<td>58</td>
<td>Monotherapy</td>
<td>100%</td>
<td>20 mg/day for 5 years</td>
<td>RFS</td>
<td>8.67 (1.06–71.09)</td>
<td>0.044</td>
<td>10.04 (1.17–86.27)</td>
<td>0.036</td>
<td></td>
<td></td>
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<tr>
<td>Xu et al., 2008</td>
<td>152</td>
<td>Monotherapy</td>
<td>100%</td>
<td>DDFS</td>
<td>not reported</td>
<td>4.7 (1.1–20.0)</td>
<td>0.04</td>
<td>100C/C+IM vs T/T</td>
<td></td>
<td></td>
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<tr>
<td>Okishiro et al., 2009</td>
<td>173</td>
<td>Monotherapy or +chemotherapy and/or goserelin</td>
<td>42.2%</td>
<td>20 mg/day, median 52 months</td>
<td>RFS</td>
<td>0.94 (0.34–2.60)</td>
<td>0.95</td>
<td>0.60 (0.18–1.92)</td>
<td>0.39</td>
<td>100C/C+IM vs T/T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schroth et al., 2009</td>
<td>1,325</td>
<td>Monotherapy</td>
<td>100%</td>
<td>for 5 years</td>
<td>RFS</td>
<td>1.49 (1.12–2.00)</td>
<td>0.006</td>
<td>1.04 (1.04–1.90)</td>
<td>0.03</td>
<td>wt/wt vs wt/*4/*4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bijl et al., 2009</td>
<td>85</td>
<td>not reported</td>
<td>not reported</td>
<td>not reported</td>
<td>Breast cancer mortality</td>
<td>not reported</td>
<td>2.12 (1.28–3.50)</td>
<td>0.003</td>
<td>1.90 (1.10–3.28)</td>
<td>0.02</td>
<td>wt/wt vs PM</td>
<td></td>
</tr>
<tr>
<td>Kiyotani et al., 2010</td>
<td>282</td>
<td>Monotherapy</td>
<td>100%</td>
<td>20 mg/day for 5 years</td>
<td>RFS</td>
<td>not reported</td>
<td>4.44 (1.31–15.00)</td>
<td>0.017</td>
<td>wt/wt vs wt/*4/*4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ramón et al., 2010</td>
<td>91</td>
<td>Monotherapy or +chemotherapy</td>
<td>39.8%</td>
<td>not reported</td>
<td>DFS</td>
<td>not reported</td>
<td>9.52 (2.79–32.45)</td>
<td>0.0032</td>
<td>wt/wt vs wt/*4/*4</td>
<td></td>
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</tbody>
</table>

CI, confidence interval; RFS, recurrence-free survival; DFS, disease-free survival; EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer.

Genotype group was reassigned using reported data.


Definition of genotype groups: wt/wt, 2 wt alleles; EM; wt/wt or wt/im; hetEM/IM, wt/im, wt/pm, im/im or im/pm; PM, 2 pm alleles; decreased, wt/pm, im/im, im/pm or pm/pm.

Log-lank test p value.

Genotype group defined as combination of CYP2D6 *4 and CYP2D6 inhibitors by Goetz et al.42.

These studies included patients reported previously.41,42,43

Genotype group as defined using reported data.
of 486 postmenopausal patients (206 of them received adjuvant tamoxifen). In 2009, Schroth et al. subsequently published a retrospective analysis of 1,325 German and North American breast cancer patients who were at an early stage and treated with adjuvant tamoxifen, and observed that PMs revealed a higher risk of recurrence than EMs with HR of 1.90 for a time to recurrence (p = 0.02); however, no significant difference in overall survival was observed. In Asians, Kiyotani et al. reported that CYP2D6*10 was significantly associated with shorter RFS in Japanese patients receiving adjuvant tamoxifen monotherapy in 2008 (HR, 10.04; p = 0.036), and also confirmed significant association in a follow-up study of 282 Japanese patients receiving adjuvant tamoxifen monotherapy (HR, 9.52; p = 0.000036 for RFS). The worse clinical outcome of tamoxifen therapy in the patients carrying CYP2D6*10 was also confirmed in a Chinese population. Although still based on retrospective analyses of tumor samples, the majority of these trials suggest that the presence of one or two variant CYP2D6 alleles is associated with shorter RFS. However, several studies have reported discordant results. Two large retrospective studies reported an inverse association between CYP2D6 genotype and breast cancer outcomes. Nowell et al. reported a trend toward better overall survival with HR of 0.77 in a cohort of adjuvant tamoxifen-treated breast cancer patients with the CYP2D6*4 genotype. A Swedish trial reported the better outcome for patients with at least one CYP2D6*4 allele who were treated with 40 mg of adjuvant tamoxifen for 2 years. An independent and larger cohort study by the same group also suggested that women with ER-positive tumors who were homozygous for CYP2D6*4 revealed no significant difference in DFS compared with those with CYP2D6*1.

There may be several reasons for these discrepancies among the studies showing the positive and negative associations. As several reviews have pointed out, considerable heterogeneity in sample collection or analysis among the studies described as follows makes it hard to compare them simply: 1) differences in dosage and duration of tamoxifen treatment, 2) incompleteness of allele determination, especially for CYP2D6*5 allele, and most importantly 3) selection of study participants. Several reports assessed partly these confounding factors. We reported significant effects of CYP2D6 genotypes on shorter recurrence-free survival only in patients with the tamoxifen monotherapy (p = 0.000036) but not in those with the combination chemotherapy (p = 0.53) as previous publications support this notion. In addition, the importance of wide coverage of CYP2D6 alleles was clearly demonstrated by Schroth et al. They reported that by increasing genotyping coverage, HR for RFS and the associated power were increased.

Overall, many reports investigated the association of CYP2D6 genotype and plasma concentration of endoxifen, and consistently clarified that patients carrying the CYP2D6 genotype which decreased or impaired CYP2D6 function showed lower plasma levels of endoxifen than those having the homozygous wild-type genotype. For association with clinical outcome, some, but not all, of the studies showed worse clinical outcome in breast cancer patients with CYP2D6 variant alleles who were treated with tamoxifen. However, two large studies showed no association between the CYP2D6 genotype and clinical outcome, which has raised concern about the CYP2D6 genotype as a biomarker to predict tamoxifen efficacy. Genotypic Polymorphisms in Other Drug-metabolizing Enzymes and Clinical Outcome of Tamoxifen Therapy

Other CYPs, UGTs and SULTs are involved in the metabolism of tamoxifen. Hence, there is a possibility that genetic variations in these genes may affect the efficacy or toxicity of tamoxifen therapy. The most important CYP isoforms are CYP3A4 and CYP3A5, which are involved in the metabolism of more than 40% of drugs. Several polymorphisms in the CYP3A4 gene have been reported (http://www.cypalleles.ki.se/cyp3a4.htm), but their contribution may be small due to their low allelic frequencies. In contrast, genetic polymorphisms, particularly a CYP3A5*3 allele, define much of the variation of CYP3A5 expression. The frequency of the CYP3A5*3 allele is higher in Caucasians (85–95%) than in Asians (74–77%). Although several studies investigated the association of CYP3A5*3 with tamoxifen metabolism or clinical outcome of tamoxifen therapy, no significant association was observed. In CYP2C9, which catalyzes 4-hydroxylation of tamoxifen, more than 30 alleles have been reported (http://www.cypalleles.ki.se/cyp2c9.htm). Among them, CYP2C9*2 and CYP2C9*3 have been well investigated. These two alleles are present in approximately 35% of Caucasian individuals, but are much less common in Asian populations. The carriers of CYP2C9*2 or CYP2C9*3 showed significantly lower concentrations of endoxifen and 4-hydroxytamoxifen, but no significant association with clinical outcome of tamoxifen therapy was observed. For the CYP2C19 gene, CYP2C19*2 and CYP2C19*3 are null alleles. CYP2C19*2 is observed in 10–20% of Caucasians and in more than 20% of Asians. In contrast, CYP2C19*3 is very rare in Caucasians, but is relatively high at 5–10% in Asian populations. As a result, in Caucasians, the frequency of PMs related to CYP2C19 is 3%, whereas the PM frequency in Asian populations is as high as 23%. Recently, a new genetic variant in the promoter region of the CYP2C19 gene, CYP2C19*17, which was associated with increased CYP2C19 activity in vitro (UM phenotype), was identified. The frequencies of CYP2C19*17 were reported to be 18–27% in Caucasian populations and 1–4% in Asians. Schroth et al. found significant association with clinical outcome of tamoxifen treatment in carriers of...
were not in the carriers of CYP2C19*2 or CYP2C19*3. As to tamoxifen metabolism, no significant
association was reported in CYP2C19 polymorphisms.

Several investigations on SULT1A1, which causes reduced SULT1A1 activity, found no clear association with
tamoxifen efficacy nor with tamoxifen metabolism. Recent reports by Gjerde et al. addressed the association of
the SULT1A1 genotype, including copy number variation, with tamoxifen metabolism. They clarified that neither
SULT1A1 genotypes nor copy numbers influence the plasma concentration of tamoxifen and its metabolites. However,
further analysis which takes into consideration the allele copy number of SULT1A1 is required, as demonstrated in
the case of CYP2D6.

Genetic Polymorphisms in Drug Transporters and Clinical Efficacy of Tamoxifen Therapy

Although the biotransformation of tamoxifen to endoxifen has been well studied and documented as described above,
there have been few reports investigating the involvement of drug transporters in the disposition of tamoxifen and its
active metabolites, 4-hydroxytamoxifen and endoxifen.

ABCB1 (P-glycoprotein, MDR1) is an ATP-dependent,
eflux transporter with broad substrate specificity widely
appreciated for its role in mediating cellular resistance to many anticancer agents. A number of investigators
have performed clinical studies to reveal the relationship between drug pharmacokinetics and ABCB1 polymorphisms.
A synonymous single nucleotide polymorphism (SNP) 3435C>T was reported to be associated with higher digoxin
levels after oral administration. Several groups performed screenings for ABCB1 polymorphisms. These three
SNPs, 1236C>T, 2667G>T and 3435C>T, and their haplotypes are considered to be important in the ABCB1
function.

Callaghan and Higging reported that tamoxifen directly bound to ABCB1 and inhibited ABCB1-mediated vinblastine
transport, but cellular accumulation of tamoxifen itself was not influenced by ABCB1. As supporting this, it was
reported that N-desmethyaltamoxifen and 4-hydroxytamoxifen as well as tamoxifen were not substrates of this
transporter in the transport assay, although 4-hydroxytamoxifen showed some tendency. Recently, two studies
found that ABCB1 is involved in the transport of active tamoxifen metabolites, endoxifen and 4-hydroxytamoxifen.
In both reports, P-glycoprotein knockout mice showed a tendency toward higher serum concentration of
endoxifen than wild-type mice although the difference was not statistically significant, suggesting that ABCB1 does not
play a major role in regulating the absorption, distribution or excretion of endoxifen. With respect to the association with
the clinical outcome of tamoxifen, no single nucleotide

![Graph](image-url)

**Fig. 2.** Kaplan-Meier estimates of recurrent-free survival and steady-state plasma concentrations of endoxifen and 4-hydroxytamoxifen according to ABC2 genotype

(A) In 282 patients treated with adjuvant tamoxifen monotherapy, rs3740065 G allele was significantly associated with shorter recurrence-free survival. (B, C) Steady-state plasma concentrations of endoxifen (B) and 4-hydroxytamoxifen (C) were not significantly different among rs3740065 genotype groups.

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polymorphism (SNP), which includes the SNPs described above, showed significant association in our recent report.\(^8\)

ABCC2 (MRP2) plays an important role in the biliary excretion of conjugated drugs and xenobiotics, and also in that of some non-conjugated drugs including pravastatin and methotrexate. Tamoxifen and its metabolites are excreted into the biliary tract as glucuronides or sulfates.\(^6\) However, there has been no report investigating the involvement of ABCC2 in the transport of tamoxifen and its active metabolites. Recent SNP screening for the ABCC2 gene identified several common SNPs such as \(-1774\text{del}G\, (\ast 1A), \)-24C>T\, (\ast 1C) and 1249G>A\, (\ast 2).\(^8\) No functional significance of 1249G>A causing Val417Ile has been shown \textit{in vivo} but its \textit{in vitro} association was reported.\(^8\) In our recent study, an intronic SNP of ABCC2 (rs3740065) was found to be significantly associated with the clinical outcome of patients with tamoxifen therapy, whereas this SNP was not associated with plasma concentration of endoxifen or 4-hydroxytamoxifen, suggesting that the contribution of ABCC2 to biliary excretion of tamoxifen and its metabolites might be limited (Fig. 2).\(^8\) An \textit{in vitro} study reporting that ABCC2 was expressed at higher levels in tamoxifen-resistant breast cancer cells suggests the possibility that active metabolites of tamoxifen are transported by ABCC2 from breast cancer cells.\(^6\) As described previously,\(^7\) rs3740065A/G is in strong linkage disequilibrium \((r^2 = 0.89)\) with \(-1774G/\) delG, which was reported to be associated with decreased ABCC2 promoter activity. Although we believe that rs3740065 has potential to predict efficacy of tamoxifen treatment, further analyses, including replication study and functional analysis to identify the causative SNP, will be required.

**Conclusion**

There have been several reports on the association of CYP2D6 genotype/phenotype and clinical outcome of breast cancer patients receiving tamoxifen therapy in large numbers of subjects. The association results with tamoxifen metabolism are consistent, but still controversial in association with clinical outcome. Investigation of the combination of the CYP2D6 genotype and other genes, which did not affect tamoxifen pharmacokinetics, may be one of the important approaches to identify the prediction marker(s) for the clinical efficacy of tamoxifen.

**References**


Kazuma Kiyotani, et al.


Hoffmeier, S., Burkh, O., von Richter, O., Arnold, H. P., Brockmoller, J., John, A., Casimir, I., Gerloff, L., Roots, I.,


