

## Significant Effect of Polymorphisms in *CYP2D6* and *ABCC2* on Clinical Outcomes of Adjuvant Tamoxifen Therapy for Breast Cancer Patients

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### A B S T R A C T

#### Purpose

The clinical efficacy of tamoxifen is suspected to be influenced by the activity of drug-metabolizing enzymes and transporters involved in the formation, metabolism, and elimination of its active forms. We investigated relationships of polymorphisms in transporter genes and *CYP2D6* to clinical outcome of patients receiving tamoxifen.

#### Patients and Methods

We studied 282 patients with hormone receptor–positive, invasive breast cancer receiving tamoxifen monotherapy, including 67 patients who have been previously reported. We investigated the effects of allelic variants of *CYP2D6* and haplotype-tagging single nucleotide polymorphisms (tag-SNPs) of *ABCB1*, *ABCC2*, and *ABCG2* on recurrence-free survival using the Kaplan-Meier method and Cox regression analysis. Plasma concentrations of tamoxifen metabolites were measured in 98 patients receiving tamoxifen 20 mg/d.

#### Results

*CYP2D6* variants were significantly associated with shorter recurrence-free survival ( $P = .000036$ ; hazard ratio [HR] = 9.52; 95% CI, 2.79 to 32.45 in patients with two variant alleles v patients without variant alleles). Among 51 tag-SNPs in transporter genes, a significant association was found at rs3740065 in *ABCC2* ( $P = .00017$ ; HR = 10.64; 95% CI, 1.44 to 78.88 in patients with AA v GG genotypes). The number of risk alleles of *CYP2D6* and *ABCC2* showed cumulative effects on recurrence-free survival ( $P = .000000055$ ). Patients carrying four risk alleles had 45.25-fold higher risk compared with patients with  $\leq$  one risk allele. *CYP2D6* variants were associated with lower plasma levels of endoxifen and 4-hydroxytamoxifen ( $P = .0000043$  and  $.00052$ ), whereas no significant difference was found among *ABCC2* genotype groups.

#### Conclusion

Our results suggest that polymorphisms in *CYP2D6* and *ABCC2* are important predictors for the prognosis of patients with breast cancer treated with tamoxifen.

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### INTRODUCTION

Tamoxifen has been widely used for the treatment and prevention of recurrence in patients with estrogen receptor (ER)–positive or progesterone receptor (PR)–positive breast cancers. However, 30% to 50% of patients who receive adjuvant tamoxifen therapy experience relapse and subsequently die of the disease.<sup>1,2</sup> Despite decades of research, the mechanisms underlying the ineffectiveness in a subset of the patients are not fully understood.

Tamoxifen is a prodrug that requires metabolic activation to elicit its pharmacologic activity. It is reported that its metabolites, 4-hydroxytamoxifen and 4-hydroxy-*N*-desmethyltamoxifen (endoxifen), are the active therapeutic moieties.<sup>3-5</sup> Compared with the parent drug, these two metabolites have 100-fold greater affinity to ER and 30- to 100-fold greater potency in suppressing estrogen-dependent cell proliferation.<sup>3-5</sup> Thus, interindividual differences in the formation and elimination of these active metabolites could be one of the important factors affecting variability in response to tamoxifen.

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CYP2D6 is a key enzyme for the generation of 4-hydroxytamoxifen and endoxifen.<sup>6</sup> Several groups including ours have clarified that loss or decrease of CYP2D6 function by genetic polymorphisms is associated with poorer clinical outcome of tamoxifen treatment in different populations.<sup>7-10</sup> Although genetic polymorphisms in *CYP2D6* are one of the most important determinants for clinical efficacy of tamoxifen, individual differences in responsiveness to tamoxifen still remain, even if the effects of *CYP2D6* polymorphisms are considered, suggesting the existence of another genetic factor(s) contributing to individual differences in the formation and elimination of the active metabolites of tamoxifen. However, no large-scale clinical trial exists that investigates the relationship of polymorphisms in tamoxifen pharmacokinetics (PK)-related genes, including *CYP2D6*, to clinical efficacy of adjuvant tamoxifen therapy. Tamoxifen is primarily eliminated by hepatic oxidative metabolism; approximately 75% of the dose is excreted into the biliary tract as glucuronides.<sup>4</sup> It has been reported that several transporters expressed in cancer cells play important roles in resistance to tamoxifen.<sup>11-13</sup> Therefore, adenosine triphosphate-binding cassette (ABC) transporter B1 (*ABCB1*; also known as multidrug resistance 1 [*MDR1*]), *ABCC2* (also known as multidrug resistance-associated protein 2 [*MRP2*]), and *ABCG2* (also known as breast cancer resistance protein [*BCRP*]), which have been reported to transport various types of glucuronides or sulfates,<sup>14,15</sup> may be involved in the transport of tamoxifen and/or its metabolites, and their genetic variations may influence the individual difference in tamoxifen efficacy.

In this study, we focused on three ABC transporters and comprehensively analyzed their associations, as well as the association of *CYP2D6*, with clinical outcome after tamoxifen monotherapy, using a larger number of samples. In addition, we investigated the effects of these polymorphisms on the steady-state plasma concentrations of tamoxifen and its metabolites in patients with breast cancer receiving tamoxifen therapy.

## PATIENTS AND METHODS

### Patients

We had two objectives in this study. The primary objective was to determine the association between genotype of the tamoxifen PK-related genes and recurrence-free survival (genotype-efficacy study). The secondary objective was to determine the association between genotype of the tamoxifen PK-related genes and plasma concentrations of tamoxifen and its metabolites (genotype-PK study) in patients with breast cancer receiving tamoxifen therapy. In the genotype-efficacy study, 282 patients with primary breast cancer (including 67 patients who have been reported previously<sup>7</sup>) were recruited from September 2007 to April 2009 at Shikoku-\*10 collaborative group (Tokushima Breast Care Clinic, Yamakawa Breast Clinic, Shikoku Cancer Center, Kochi University Hospital, and Itoh Surgery and Breast Clinic), Kansai Rosai Hospital, Sapporo Breast Surgical Clinic, and Sapporo Medical University Hospital. All patients were Japanese women pathologically diagnosed with ER- and/or PR-positive, invasive breast cancer without distant spread who received adjuvant tamoxifen monotherapy without any other treatments after surgical treatment between 1986 and 2007. Data on primary breast cancer diagnosis or recurrence were confirmed from the patients' medical records. Patients without recurrence were censored at the date of the last consultation. Recurrence-free survival time was defined as the time from surgical treatment to diagnosis of the recurrence of a breast cancer (locoregional, distant metastasis, and contralateral breast events). Patients received tamoxifen 20 mg/d for 5 years; tamoxifen was stopped at the time a recurrence was identified. ER and PR status was evaluated by enzyme immunoassay or immunohistochemistry. The

cutoff for human epidermal growth factor receptor 2 overexpression was defined as 3+ immunohistochemical staining.<sup>16</sup> Nodal status was determined according to the International Union Against Cancer TNM classification. For the genotype-PK study, 98 independent patients with breast cancer who had been taking tamoxifen 20 mg daily for at least 4 weeks (patients taking selective serotonin reuptake inhibitors were not included) were recruited at Tokushima Breast Care Clinic (Tokushima, Japan). Blood samples (5 mL) were collected before taking tamoxifen. These studies were approved by the Institutional Review Board of the Institute of Medical Science, The University of Tokyo (Tokyo, Japan), and written informed consent was obtained from all patients.

### Genotyping

Genomic DNA was extracted from peripheral blood using Qiagen DNA extraction kit (Qiagen, Valencia, CA). Genotyping for key polymorphisms for *CYP2D6\*4* (1846G>A), *CYP2D6\*6* (1707delT), *CYP2D6\*10* (100C>T), *CYP2D6\*14B* (1758G>A), *CYP2D6\*18* (4125\_4133dupGTGCCACT), *CYP2D6\*21* (2573\_2574insC), *CYP2D6\*36* (gene conversion to *CYP2D7* in exon 9), and *CYP2D6\*41* (2988G>A) was performed using previously designed primers and probes.<sup>17</sup> Determination of allelic ratio and copy number of *CYP2D6* gene was performed using ABI PRISM 7900HT (Applied Biosystems, Foster City, CA), as described previously.<sup>17,18</sup> To determine the diploypes, whole-gene deletion (*CYP2D6\*5*) and duplications (*CYP2D6\*1-1*, *CYP2D6\*10-10*, *CYP2D6\*10-36*, and *CYP2D6\*36-36*) were detected following reported protocols.<sup>19,20</sup> Multiplication alleles, which consisted of *CYP2D6\*10* and *CYP2D6\*36* (ie, *CYP2D6\*10-36* and *CYP2D6\*10-36-36*), were defined as *CYP2D6\*10* because the enzyme encoded by *CYP2D6\*36* has been reported to have negligible activity.<sup>21</sup> To evaluate the effects of all *CYP2D6* alleles tested in this study, we defined all decreased and null alleles (\*4, \*5, \*10, \*10-\*10, \*14, \*21, \*36-\*36, and \*41) as allele *V* and \*1 and \*1-\*1 alleles as allele *wt*.

For *ABCB1*, *ABCC2*, and *ABCG2*, we genotyped haplotype-tagging single nucleotide polymorphisms (tag-SNPs) selected with an  $r^2$  threshold of 0.8 and minor allele frequency of greater than 10% in the HapMap Japanese population as described in our previous study.<sup>22</sup> In addition, we genotyped the following possible functional polymorphisms: rs2032582 (2677G>T/A) and rs3213619 (-129T>C) in *ABCB1* and rs2273697 (1249G>A) in *ABCC2*. A total of 51 SNPs were genotyped using multiplex polymerase chain reaction-based Invader assay (Third Wave Technologies, Madison, WI) as described previously.<sup>23</sup>

### Measurement of Plasma Concentrations of Tamoxifen and Its Metabolites

Plasma concentrations of tamoxifen and its metabolites, *N*-desmethyltamoxifen, 4-hydroxytamoxifen, and endoxifen, were measured using a high-performance liquid chromatography with time of flight mass spectrometry (HPLC-TOFMS). Tamoxifen ( $\geq 99\%$ ), (*Z*)-4-hydroxytamoxifen ( $\geq 98\%$ ), and imipramine, an internal standard (IS), were purchased from Sigma-Aldrich (St Louis, MO). *N*-desmethyltamoxifen ( $> 98\%$ ) was obtained from Toronto Research Chemicals (North York, Ontario, Canada). Endoxifen was used as a mixture of *E* and *Z* isomers (25:75) described previously.<sup>5</sup>

Pretreatment of plasma samples was carried out by protein precipitation and solid phase extraction. Briefly, 100  $\mu$ L IS solution (imipramine 200 ng/mL in acetonitrile) was added to 100  $\mu$ L plasma. After mixing and centrifugation (13,000 rpm, 5 minutes, 4°C), 950  $\mu$ L of 25 mmol/L sodium citrate buffer (pH 5.0) was added to the supernatant. The solutions were then purified by solid phase extraction using BOND ELUTE-C18 cartridges (1 mL, 100 mg; Varian, Lake Forest, CA) following the method described previously.<sup>24</sup>

HPLC-TOFMS was equipped with a 1200 series HPLC system and a G6210 Time-of-Flight LC/MS (Agilent Technologies, Santa Clara, CA). Chromatographic separations were obtained under gradient conditions using an XBridge C18 column (150  $\times$  3 mm ID, 3.5  $\mu$ m particle size; Waters, Milford, MA). The mobile phase was consisted of eluent A (10 mmol/L ammonium formate) and eluent B (acetonitrile). The gradient was as follows: 30% B for 2.0 minutes, 0.2 mL/min; 30% B at 2.01 minutes, 0.5 mL/min; 30% B at 3.0 minutes, 0.5 mL/min, 90% B at 20 minutes, 0.5 mL/min; and 98% B at 20.01 minutes, 1.2 mL/min. Injection volume was 20  $\mu$ L, and all separations were

performed at 30°C. The retention times of imipramine (IS), endoxifen, 4-hydroxytamoxifen, *N*-desmethyltamoxifen, and tamoxifen were 10.68, 12.22, 12.89, 16.29, and 17.39 minutes, respectively. Electrospray ionization-TOFMS was conducted in the positive ion mode. The capillary voltage was set at 4,000 V. A flow rate and a nebulizer pressure of nitrogen gas (heater temperature 360°C) were maintained at 10 L/min and 45 psig, respectively. In TOFMS, the fragmenter voltage was set at 150 V. Two mass-to-charge ratio (*m/z*) values of imipramine (IS), *N*-desmethyltamoxifen, tamoxifen, endoxifen, and 4-hydroxytamoxifen were 281.2, 358.2, 372.2, 374.2, and 388.2, respectively.

Standard curves were prepared in the concentration range of 20 to 1,000 ng/mL for tamoxifen and *N*-desmethyltamoxifen, 3.75 to 187.5 ng/mL for endoxifen, and 1 to 20 ng/mL for 4-hydroxytamoxifen. The interday and intraday variability in precision (expressed as the coefficient of variation) for all compounds ranged from 0.7% to 6.6% and from 5.5% to 9.5%, respectively; the average accuracies were between 94.1% and 102.9%.

### Statistical Analysis

Recurrence-free survival curves were estimated using the Kaplan-Meier method. Statistical significance of a relationship between clinical outcome and genetic polymorphism was assessed using the trend log-rank test. Cox proportional hazards analysis was used to identify significant prognostic clinical factors and to test for an independent contribution of genetic factors to recurrence-free survival. To examine potential confounding, age was treated as

a continuous variable, tumor size was treated as an ordinal variable, and the other covariates were treated as categorical variables. Genotypes were analyzed by assigning an ordinal score to each genotype (1 for homozygous nonrisk allele, 2 for heterozygous risk allele, and 3 for homozygous risk allele). The significant subsets of variables in the univariate analysis were used in multivariate analysis. All polymorphisms evaluated in this study were tested for deviation from Hardy-Weinberg equilibrium with the use of a  $\chi^2$  test. Risk alleles were defined as the alleles associated with shorter recurrence-free survival. Combination effects were investigated by adding up the number of risk alleles of *CYP2D6* and *ABCC2* genes. The differences in plasma concentrations of tamoxifen and its metabolites among genotypes were evaluated by a Kruskal-Wallis test. Statistical tests provided two-sided *P* values, and a significance level of *P* < .05 was used. In the screening of transporter genes, we used a significance level of *P* < .00098 to adjust multiple testing by the strict Bonferroni's correction. Statistical analyses were carried out using SPSS (version 17.0; SPSS, Chicago, IL) and the R statistic program (<http://www.r-project.org/>).

## RESULTS

### Patient Characteristics

To evaluate the effect of tamoxifen on recurrence-free survival, we recruited 282 Japanese patients receiving adjuvant tamoxifen monotherapy without any other anticancer treatments in this study. Table 1 lists the characteristics of these 282 patients who were pathologically diagnosed to have an ER- and/or PR-positive, invasive breast cancer. Their median age at the time of surgery was 51 years old (range, 31 to 83 years), and the median follow-up period was 7.1 years (range, 0.8 to 23.5 years). Among the characteristics listed in Table 1, tumor size and nodal status showed significant associations with recurrence-free survival (*P* = .037 and .049, respectively) in the Cox proportional hazards analysis.

### Associations Between Genotypes and Clinical Outcome

We determined *CYP2D6* genotypes of these 282 patients (Table 2). The allele frequency of *CYP2D6*\*10, which is considered to have decreased enzymatic activity and is known to be present at a relatively high frequency in Asian populations, was 37.2%. The frequencies of the alleles observed in this study were comparable to those reported previously.<sup>17,20</sup> To evaluate the effects of the *CYP2D6* alleles, we examined all of the decreased and null alleles (\*4, \*5, \*10, \*10-\*10, \*14, \*21, \*36-\*36, and \*41) as allele *V* and \*1 and \*1-\*1 alleles as allele *wt*. Kaplan-Meier estimates indicated that the patients carrying one or two variant alleles (*wt/V* or *V/V*) had significantly shorter recurrence-free survival compared with patients with homozygous wild-type alleles (*wt/wt*; log-rank *P* = .0002; Fig 1A). In Cox proportional hazards analysis, the *CYP2D6* genotype was an independent indicator of recurrence-free survival after adjustment of tumor size and nodal status (trend *P* = .000036, Table 3). The adjusted hazard ratios (HRs) of patients with *wt/V* and *V/V* genotypes, relative to patients with *wt/wt*, were 4.44 (95% CI, 1.31 to 15.00) and 9.52 (95% CI, 2.79 to 32.45), respectively.

We also genotyped tag-SNPs in ABC transporter genes, *ABCB1*, *ABCC2*, and *ABCG2*, which are suspected to be involved in the biliary excretion of tamoxifen or its metabolites (Appendix Table A1, online only).<sup>15</sup> Among 51 SNPs tested, five SNPs in *ABCC2* showed *P* < .05 by log-rank test for recurrence-free survival. The lowest *P* value was observed at rs3740065 in *ABCC2* (log-rank *P* = .0002, Fig 1B), which was significant after the adjustment for multiple testing by the strict

**Table 1.** Patient Demographics and Clinical Characteristics

Demographic or Clinical Characteristic	No. of Patients (N = 282)	%
Age at surgery, years		
Median	51	
Range	31-83	
Menopausal status		
Premenopausal	123	43.6
Postmenopausal	149	52.8
Unknown	10	3.6
Tumor size, cm		
≤ 2	159	56.4
2.1-5	106	37.6
> 5	2	0.7
Unknown	15	5.3
Nodal status		
Negative	230	81.6
Positive	48	17.0
Unknown	4	1.4
ER status		
Positive	208	73.8
Negative	25	8.9
Unknown	49	17.3
PR status		
Positive	195	69.1
Negative	36	12.8
Unknown	51	18.1
HER2		
Positive*	5	1.8
Negative	97	34.4
Unknown	180	63.8
Events		
No event	241	85.5
Locoregional events	9	3.2
Distant metastasis events	22	7.8
Contralateral breast events	10	3.5

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.  
\*Score of 3+ in immunohistochemistry.

**Table 2.** Genotype Frequency of *CYP2D6*

Combined <i>CYP2D6</i> genotype†	No. of Patients	%
<i>wt/wt</i>	83	29.4
*1/*1		
<i>wt/V</i>	105	37.2
*1/*4		
*1/*5		
*1/*10		
*1/*10*10		
*1/*14		
*1/*21		
*1/*36*36		
*1/*41		
*1*1/*10		
<i>V/V</i>		
*5/*5		
*5/*10		
*5/*21		
*5/*41		
*10/*10		
*10/*10*10		
*10/*21		
*10/*36*36		
*10/*41		

†All decreased or null alleles (\*4, \*5, \*10, \*10\*10, \*14, \*21, \*36\*36, and \*41) were defined as an allele V, and \*1 and \*1\*1 alleles were defined as wt.

Bonferroni's correction. rs11190303 in *ABCC2* also showed a significant association (log-rank  $P = .00048$ ), which is in strong linkage disequilibrium (LD) with rs3740065 ( $r^2$  of LD = 0.79). No significant associations between genotypes and recurrence-free survival were observed for the remaining SNPs. Cox proportional hazards analysis revealed that rs3740065 in *ABCC2* was an independent indicator of recurrence-free survival (trend  $P = .00017$ , Table 3) and that the adjusted HR for patients carrying rs3740065 AA, compared with patients carrying GG genotype as a reference, was 10.64 (95% CI, 1.44 to 78.88; Table 3), indicating rs3740065 A to be a risk allele of recurrence.

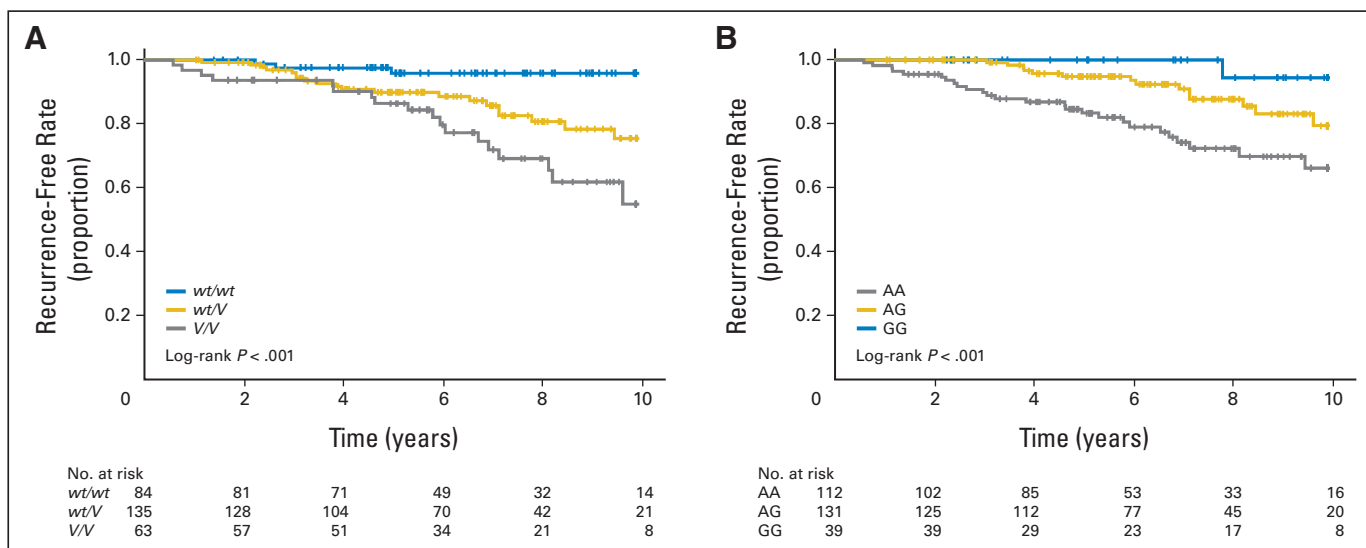
We then investigated a combined effect of *CYP2D6* and *ABCC2* genotypes on the recurrence-free survival by classifying the patients into five groups (zero, one, two, three, and four risk allele groups) according to the number of risk alleles of *CYP2D6* and *ABCC2* genes. Kaplan-Meier analysis revealed the number of risk alleles of these two genes to have cumulative effects on recurrence-free survival (log-rank  $P = .000000083$ , Fig 2). Adjusted HRs for risk of recurrence computed for patients carrying two or more risk alleles increased from 4.93-fold (two risk alleles) to 45.25-fold (four risk alleles; trend  $P = .000000055$ , Table 3).

### Associations Between Genotypes and Plasma Concentrations

To analyze the effects of *CYP2D6* and *ABCC2* genotypes on the plasma concentrations of tamoxifen and its metabolites, we recruited 98 independent patients with breast cancer taking tamoxifen 20 mg/d as an adjuvant therapy. The steady-state plasma concentrations of endoxifen among the three genotypic groups of *wt/wt*, *wt/V*, and *V/V* were significantly different ( $P = .0000043$ , Fig 3A). Median plasma concentrations of endoxifen in patients with *V/V* and *wt/V* were 15.5 and 27.2 ng/mL, respectively, which were 43.8% and 76.8% of the concentration in patients with *wt/wt* (35.4 ng/mL). The differences in plasma 4-hydroxytamoxifen concentrations among *CYP2D6* genotype groups were also statistically significant ( $P = .00052$ , Fig 3C). When compared among three genotype groups for *CYP2D6*\*10, similar results were observed (Appendix Fig A1, online only). However, there was no significant association between genotypes of *ABCC2* rs3740065 and plasma concentrations of endoxifen and 4-hydroxytamoxifen (Figs 3B and 3D). Plasma tamoxifen and *N*-desmethyltamoxifen levels were also not significantly different among the three genotype groups (data not shown).

## DISCUSSION

Adjuvant tamoxifen treatment substantially improves the 10-year survival of patients with ER-positive breast cancer, with a significant



**Fig 1.** Kaplan-Meier estimates of recurrence-free survival for *CYP2D6* and *ABCC2* rs3740065 genotypes. (A) Comparison of 282 patients with *wt/wt*, *wt/V*, and *V/V* of *CYP2D6*. (B) Comparison of 282 patients with AA, AG, and GG genotypes at rs3740065 in *ABCC2*.

**Table 3.** Recurrences and Hazard Ratios for Recurrence-Free Survival in Patients With Breast Cancer Treated With Tamoxifen

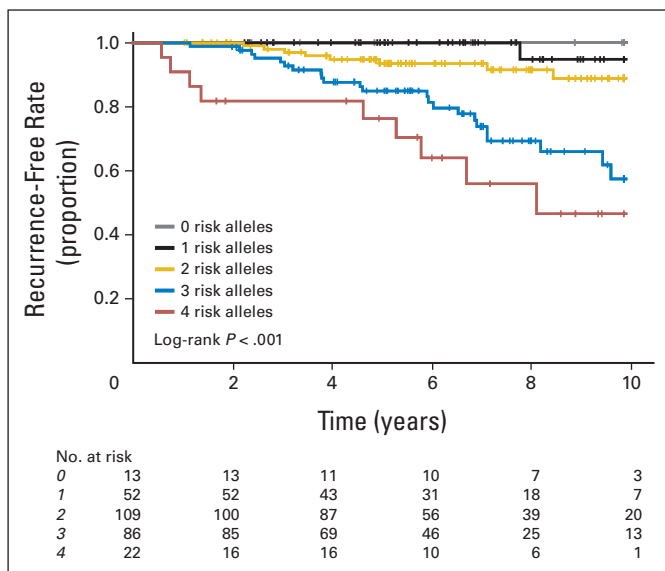
Variable	No. of Patients	No. of Recurrences	Adjusted Hazard Ratio*	95% CI	P (trend test)
<i>CYP2D6</i> genotype					
wt/wt	84	3	1.00 (reference)		.000036
wt/V	135	20	4.44	1.31 to 15.00	
V/V	63	18	9.52	2.79 to 32.45	
<i>ABCC2</i> rs3740065					
GG	39	1	1.00 (reference)		.00017
AG	131	14	3.52	0.46 to 26.79	
AA	112	26	10.64	1.44 to 78.88	
No. of risk alleles of <i>CYP2D6</i> and <i>ABCC2</i> rs3740065					
0	13	0	1.00 (reference)†		.000000055
1	52	1			
2	109	8	4.93	0.61 to 39.63	
3	86	23	19.98	2.69 to 148.65	
4	22	9	45.25	5.58 to 366.81	

\*Adjusted for tumor size and nodal status.  
 †The reference category included ≤ one risk allele.

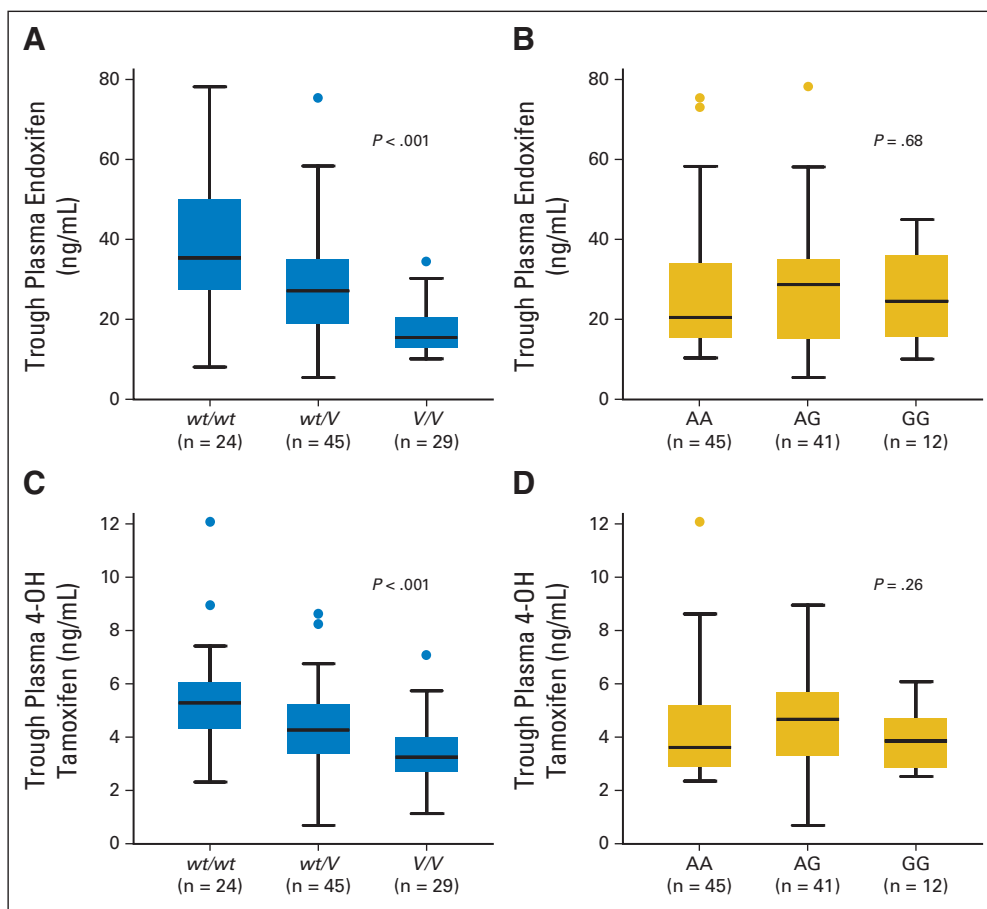
reduction in breast cancer recurrence and mortality.<sup>2</sup> Although aromatase inhibitors have demonstrated superiority to tamoxifen as adjuvant therapy for early breast cancer in postmenopausal women, there are some reports indicating that aromatase inhibitors are associated with a higher risk of osteoporosis than tamoxifen.<sup>25,26</sup> Therefore, tamoxifen still plays a major therapeutic role in ER-positive breast cancer. We previously reported that *CYP2D6\*10* was significantly associated with clinical outcome in 67 patients with breast cancer receiving adjuvant tamoxifen monotherapy.<sup>7</sup> One group replicated this association; they also found a significant association between *CYP2D6\*10* and disease-free survival in 152 patients.<sup>27</sup> Lim et al<sup>28</sup> reported a significant association between *CYP2D6\*10* and time to progression for patients with metastatic breast cancer receiving tamoxifen, which supports our results. We studied additional patients

with breast cancer who received tamoxifen monotherapy and confirmed the significant associations of *CYP2D6\*10* with recurrence-free survival in the additionally collected sample set (n = 175; log-rank P = .0048). When sample sets of the previous and current studies were combined, the patients with *CYP2D6\*1/\*10* and *CYP2D6\*10/\*10* showed significantly shorter recurrence-free survival compared with patients with *CYP2D6\*1/\*1* (log-rank P = .00027, Appendix Fig A1). We also clarified that *CYP2D6\*10* was associated with lower plasma levels of endoxifen using 98 patients with breast cancer who were receiving tamoxifen therapy (Appendix Fig A1). These results suggest that a lower clinical efficacy in the tamoxifen-treated patients with *CYP2D6\*10/\*10* may be caused by lower systemic exposure to endoxifen.

We found that two SNPs in *ABCC2* were significantly associated with recurrence-free survival of the patients receiving tamoxifen monotherapy. *ABCC2* is a member of the ABC transporter, which is involved in the transport of many kinds of drugs and glucuronide conjugates. An in vitro study reporting that *ABCC2* was expressed at higher levels in tamoxifen-resistant breast cancer cells suggested that active metabolites of tamoxifen were transported by *ABCC2* from breast cancer cells.<sup>29</sup> Although we measured steady-state plasma concentrations of tamoxifen and its metabolites, we could not find significant differences among the *ABCC2* rs3740065 genotype groups (Fig 3). From these lines of evidence, we suspect that *ABCC2* might regulate local exposure of endoxifen to breast cancer cells. The SNP most significantly associated with recurrence-free survival (rs3740065) in this study was one of the tag-SNPs located in intron 29 of the *ABCC2* gene. Although we also genotyped one nonsynonymous SNP rs2273697 (I417V),<sup>30</sup> we could not find any association of this SNP with recurrence-free survival (log-rank P = .88, r<sup>2</sup> of LD with rs3740065 = 0.05). Therefore, we speculate that rs3740065 SNP or some other genetic variations linked to rs3740065 in *ABCC2* may be associated with increased expression levels or transport activity of *ABCC2* in breast cancer tissue, causing the lower exposure of breast cancer cells to endoxifen. However, further functional analysis will be needed to clarify the biologic mechanisms associated with clinical outcome of tamoxifen therapy.



**Fig 2.** Kaplan-Meier estimates of recurrence-free survival for combination effects of *CYP2D6* and *ABCC2* genotypes. The patients were classified into five groups (zero, one, two, three, or four alleles) based on the number of risk alleles of these two genes.



**Fig 3.** Steady-state plasma concentrations of (A, B) endoxifen and (C, D) 4-hydroxytamoxifen (4-OH tamoxifen) according to genotypes. (A, C) Comparison among *wt/wt*, *wt/V*, and *V/V* of *CYP2D6*. (B, D) Comparison among AA, AG, and GG genotypes at rs3740065 in *ABCC2*. The horizontal line indicates the median concentration, the box covers the 25th to 75th percentiles, and the maximum length of each whisker is  $1.5 \times$  the interquartile range; dots outside the whiskers are outliers.

In summary, our results indicate that genetic polymorphisms in *CYP2D6* and *ABCC2* may be important predictors for the clinical outcome of tamoxifen treatment for patients with breast cancer. These findings have the potential to improve the ability of physicians to select optimal hormonal therapy for the treatment of hormone receptor-positive breast cancer.

#### AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

#### AUTHOR CONTRIBUTIONS

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