

Breast Cancer Treatment Outcome With Adjuvant Tamoxifen Relative to Patient CYP2D6 and CYP2C19 Genotypes

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A B S T R A C T

Purpose

The clinical outcome of tamoxifen-treated breast cancer patients may be influenced by the activity of cytochrome P450 enzymes that catalyze the formation of antiestrogenic metabolites endoxifen and 4-hydroxytamoxifen. We investigated the predictive value of genetic variants of CYP2D6, CYP2C19, and three other cytochrome P450 enzymes for tamoxifen treatment outcome.

Patients and Methods

DNA from 206 patients receiving adjuvant tamoxifen monotherapy and from 280 patients not receiving tamoxifen therapy (71 months median follow-up) was isolated from archival material and was genotyped for 16 polymorphisms of CYP2D6, CYP2C19, CYP2B6, CYP2C9, and CYP3A5 by matrix-assisted, laser desorption/ionization, time-of-flight mass spectrometry, and by copy number quantification. Risk and survival estimates were calculated using logistic regression, Kaplan-Meier, and Cox regression analyses.

Results

Tamoxifen-treated patients carrying the CYP2D6 alleles *4, *5, *10, *41—all associated with impaired formation of antiestrogenic metabolites—had significantly more recurrences of breast cancer, shorter relapse-free periods (hazard ratio [HR], 2.24; 95% CI, 1.16 to 4.33; $P = .02$), and worse event-free survival rates (HR, 1.89; 95% CI, 1.10 to 3.25; $P = .02$) compared with carriers of functional alleles. Patients with the CYP2C19 high enzyme activity promoter variant *17 had a more favorable clinical outcome (HR, 0.45; 95% CI, 0.21 to 0.92; $P = .03$) than carriers of *1, *2, and *3 alleles.

Conclusion

Because genetically determined, impaired tamoxifen metabolism results in worse treatment outcomes, genotyping for CYP2D6 alleles *4, *5, *10, and *41 can identify patients who will have little benefit from adjuvant tamoxifen therapy. In addition to functional CYP2D6 alleles, the CYP2C19 *17 variant identifies patients likely to benefit from tamoxifen.

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INTRODUCTION

The clinical benefit of the antiestrogen agent tamoxifen for the treatment of estrogen receptor (ER)-positive breast cancer has been evident for more than three decades by the eminent reduction of recurrence (47%) and mortality rates (26%).¹ Yet, 30% to 50% of patients with adjuvant tamoxifen therapy relapse or die. Although this may be explained in part by tumor-dependent ER activation² and downstream pathways classified by molecular profiles,³⁻⁵ it has become increasingly clear that treatment efficacy is considerably influenced by intrinsic host factors.

Several lines of evidence suggest that the metabolites 4-hydroxytamoxifen (4-OH-TAM) and 4-hydroxy-*N*-desmethyltamoxifen (endoxifen) are the active therapeutic moieties. Compared with the parent drug, these two metabolites have at least 100-fold higher potency, in terms of binding to ER and of suppression of breast cancer cell proliferation.⁶⁻¹⁰ One of the principal mechanisms of tamoxifen-induced tumor cell growth inhibition is the activation of antiproliferative, transforming growth factor-beta (TGF- β) signal transduction pathways. Accordingly, the degree of tumor cell growth inhibition has been shown to be exclusively mediated by 4-OH-TAM and endoxifen but not by other metabolites, and,

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Ulrich M. Zanger is a named coinventor of pending patent applications directed to the detection of CYP2D6 *41 allele polymorphisms for diagnostic purposes and is entitled to share in any net income derived from licensing these patent rights under standard academic institutional policies.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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moreover, was related to an upregulation of TGF- β secretion and TGF- β receptors.¹¹ Thus, interindividual differences in the formation of these active metabolites could be an important source of variability in the response to tamoxifen.

The cytochrome P450 enzyme CYP2D6 is one of the key enzymes for the formation of 4-OH-TAM and endoxifen,^{9,12,13} and loss of functional alleles of CYP2D6 leads to a poor-metabolizer (PM) phenotype. This phenotype has very low endoxifen plasma levels¹⁴ and a nonfavorable clinical outcome.^{15,16} Moreover, formation of the antiestrogenic metabolites is affected by drugs, such as selective serotonin reuptake inhibitors (SSRIs) used to treat hot flashes, that inhibit CYP2D6.^{8,14,16,17} Previous studies of the relationship between CYP2D6 genotypes and tamoxifen-related clinical outcomes have been restricted to the major PM allele *4, possibly leading to inaccurate genotype-phenotype assignments, because only 60% to 70% of the 7% to 10% PMs present in a European population may have been detected. The lack of a comprehensive assessment of the known CYP2D6 deficiency variants¹⁸⁻²⁰ might explain the controversial data arising from these studies.^{15,16,21-23} Furthermore, in addition to PMs, the intermediate-metabolizer phenotype (IM), which occurs with a frequency of at least 10%, has an impaired CYP2D6 function that leads to decreased endoxifen plasma levels, similar to PMs.¹⁴ Hence, between 15% and 20% of European patient populations carry genetic CYP2D6 variants associated with a pronounced impairment in the formation of antiestrogenic tamoxifen metabolites.

The current study investigated the role of CYP2D6 variants in the outcome of adjuvant tamoxifen therapy by a comprehensive PM- and IM-genotyping approach. In addition, we analyzed CYP3A5, CYP2B6, CYP2C9, and CYP2C19 variants because these enzymes contribute to the formation of antiestrogenic metabolites.

PATIENTS AND METHODS

Patients

Formalin-fixed, paraffin-embedded tumor specimens of 621 patients diagnosed with primary invasive breast cancer between 1986 and 2000 were obtained from the archival database at the Robert-Bosch Hospital Breast Center, Stuttgart, Germany. Inclusion criteria were based on the availability of clinical follow-up information and the availability of noncancerous breast tissue sufficient for DNA extraction. Relapse-free women with a follow-up of less than 8 months were excluded from the study. We identified three treatment groups based on patient records. The adjuvant monotherapy-tamoxifen treatment group (mTAM) included 206 ER-positive patient cases. There were 280 patients who did not receive tamoxifen treatment, but who received either adjuvant chemotherapy or no drug therapy, and who served as a control group (noTAM). Patients receiving adjuvant tamoxifen and concomitant chemotherapy or patients with an unclear ER status (n = 135) were excluded from the analysis. Clinical characteristics of patients in the mTAM and noTAM groups are given in Table 1. The median age at the time of surgery was 60 years (range, 29 to 92 years), and the median postsurgery follow-up period was 71 months (range, 4 to 227 months). Because information on comedication of patients receiving SSRIs was incomplete, it was not included in the analyses. The use of archival patient materials was approved by the local ethics committee of the University Tuebingen.

Genotyping

After histologic inspection, DNA was extracted from normal breast tissue using standard procedures. For genotyping, we used matrix-assisted, laser desorption/ionization, time-of-flight mass spectrometry (MALDI-TOF MS), as described in Jaremko et al.²⁴ The CYP2D6 gene duplication and the *5 deletion alleles were determined using a TaqMan, real-time quantification assay, according to Schaeffeler et al.²⁵ The following single nucleotide polymorphisms (SNPs) at CYP2D6 and CYP2C19 were analyzed: CYP2D6 *4, *5, *10, *41, and gene duplication; and CYP2C19 *2, *3, and *17 (-806C > T and -3402C > T). We also included other genes and polymorphisms of the

Table 1. Patient and Tumor Characteristics

| Characteristic | mTAM* (n = 206) | | noTAM* (n = 280) | |
|-------------------|-----------------|------|------------------|------|
| | No. of Patients | % | No. of Patients | % |
| Age, years | | | | |
| Median | 68.4 | | 56.1 | |
| Range | 40.1 to 91.8 | | 28.7 to 88.1 | |
| Follow-up, months | | | | |
| Median | 76.6 | | 68.9 | |
| Range | 8.1 to 227.2 | | 4.3 to 198.6 | |
| Tumor size, cm | | | | |
| ≤ 2 | 92 | 45.1 | 112 | 40.6 |
| 2-5 | 94 | 46.1 | 128 | 46.4 |
| > 5 | 18 | 8.8 | 36 | 13.0 |
| Nodal status | | | | |
| N0 | 129 | 69.4 | 148 | 55.6 |
| N1 | 52 | 28.0 | 111 | 41.7 |
| N2 | 5 | 2.6 | 7 | 2.7 |
| Grading | | | | |
| G1 | 18 | 8.8 | 17 | 6.1 |
| G2 | 169 | 82.4 | 166 | 59.7 |
| G3 | 18 | 8.8 | 95 | 34.2 |
| ER status | | | | |
| Positive | 206 | 100 | 147 | 53.8 |
| Negative | — | — | 126 | 46.2 |

Abbreviations: mTAM, patients receiving adjuvant tamoxifen monotherapy; noTAM, patients receiving non-tamoxifen-containing regimens; ER, estrogen receptor. *Differences in numbers refer to unavailable information.

primary tamoxifen biotransformation in our analyses. These were CYP3A5 *3, CYP2B6 (785A > G, -82T > C, 1459C > T, and 516G > T), and CYP2C9 *2 and *3 (Appendix Table A1, online only). Base numberings and allele definitions are in accord with the CYP Allele Nomenclature Committee (<http://www.cypalleles.ki.se>). All primer sequences are available on request.

The CYP2D6-metabolizer status was defined as extensive (EM), based on the absence of *4 or *5 null alleles; heterozygote-extensive (hetEM), based on the presence of one null allele; or poor (PM or IM), based on either being homozygous for null alleles or having a combined null/IM status as defined by null/*10 or null/*41. For statistical reasons, individuals with duplicated genes were included in the EM group, provided that they were not carriers of null alleles. Because polymerase chain reaction–amplification rates were poor (50% to 60%) due to difficulties in designing specific primers that span short fragments (100 to 200 bp), the CYP2D6 PM-alleles *3, *6, *7, and *8 were not included in our analyses.

End Points and Statistical Analyses of Associations

We tested for an association between genetic variants in tamoxifen-bioactivating enzymes and relapse-free time (RFT), event-free survival (EFS), and overall survival (OS). RFT was defined as the time from surgery to the occurrence of a breast event (ie, local or distant recurrence or contralateral breast cancer). EFS was defined as the time from surgery to the occurrence of either local or distant recurrence, contralateral breast cancer, or death from any cause. OS was defined as the time from surgery to death from any cause. Patients who were alive and were without a breast event or with a second, nonbreast, primary cancer were censored at the date of the last follow-up inquiry. Risk estimates of an association between allele or genotype and occurrence of a breast event were calculated, and *P* values were adjusted for multiple comparisons. Empiric *P* values (P_{emp}) for differences in allele frequencies were generated by permuting the whole data set 1,000 times with a random assignment of outcome affection status. For SNPs that passed the multiple comparison test, odds ratios (ORs) together with 95% CIs were subsequently adjusted for prognostic clinical factors in multivariate logistic regression (OR_{adj}). Time-to-relapse and survival data were analyzed by calculating Kaplan-Meier distributions. Statistical significance of a relationship between outcome and each of the genetic polymorphisms was assessed by log-rank test. Multivariate Cox regression was used to adjust for prognostic clinical factors and to test for an independent contribution of genetic factors to the outcome variable, assuming an additive genetic model. All *P* values were two-sided, and values less than .05 were considered statistically significant. Statistical tests were run using SPSS software version 12.1 (SPSS Inc, Chicago, IL) and PLINK v0.99n (CHGR Massachusetts General Hospital, and Broad Institute of Harvard and Massa-

chusetts Institute of Technology; <http://www.pngu.mgh.harvard.edu>) for permutation procedures.

RESULTS

Allele Frequencies

Allele frequencies were calculated, and tests on deviation from the Hardy-Weinberg equilibrium were performed (Appendix Table A1, online only). Two polymorphisms, CYP2D6 *10 and CYP2C9 *2, were not in Hardy-Weinberg equilibrium. Observed allele frequencies matched those reported for populations of European descent.

Associations Between Genotypes and Clinical Outcome

There were no significant correlations between genotypes and tumor size, nodal status, histologic grade, or ER status in either mTAM or noTAM groups. Stratification for radiation treatment did not influence genotype frequencies. Cox regression analyses showed that tumor size and nodal status were significantly correlated to EFS (not shown). In subsequent analyses, these clinical parameters were used to adjust for confounding effects in multivariate analyses of genotype-outcome associations.

Two allele variants at CYP2D6 and two allele variants at CYP2C19 passed the multiple-comparison adjustment and showed significant associations with clinical outcome in the mTAM patient group but not in the noTAM control group. CYP2D6 null alleles *4 and *5 were associated with a higher frequency of relapse (OR, 2.13; 95% CI, 1.26 to 3.63; $P_{emp} = .03$). The unadjusted OR showed a two- to three-fold increased risk of relapse for CYP2D6 genotypes with at least one null allele (Table 2). Because IM genotypes had increased relapse rates similar to PMs, IM and PM genotypes subsequently were combined. The risk of relapse was close to two-fold for carriers of the PM (*4 or *5) and the PM/IM (null/*10 or null/*41) alleles (OR_{adj} , 1.86; 95% CI, 1.13 to 3.08; $P = .02$; Table 2), assuming a proportional, stepwise increase

Table 2. Frequencies and Relapse Risks for CYP2D6-Genotype–Predicted Phenotypes and for CYP2C19 *17 Genotypes in Tamoxifen-Treated Patients

| Variant | Overall | | No Event | | Event | | Unadjusted Statistics | | | Adjusted Statistics | | |
|--------------------|---------|----|----------|----|-------|----|-----------------------|---------------|----------|---------------------|--------------|----------|
| | No. | % | No. | % | No. | % | OR | 95% CI | <i>P</i> | OR_{adj} | 95% CI | <i>P</i> |
| CYP2D6 | | | | | | | | | | | | |
| homEM | 118 | 60 | 101 | 65 | 17 | 41 | 1 | | | | | |
| hetEM | 49 | 25 | 35 | 22 | 14 | 34 | 2.37 | 1.06 to 5.31 | .03 | | | |
| IM | 16 | 8 | 11 | 7 | 5 | 12 | 2.70 | 0.83 to 8.75 | .08 | | | |
| PM | 14 | 7 | 9 | 6 | 5 | 12 | 3.30 | 0.99 to 11.05 | .04 | | | |
| IM and PM Combined | | | | | | | | | | | | |
| homEM | 118 | 60 | 101 | 65 | 17 | 42 | 1 | | | | | |
| hetEM | 49 | 25 | 35 | 22 | 14 | 34 | 2.37 | 1.06 to 5.31 | .03 | 1.86 | 1.13 to 3.08 | .02 |
| PM + IM | 30 | 15 | 20 | 13 | 10 | 24 | 2.97 | 1.18 to 7.43 | .01 | | | |
| CYP2C19 | | | | | | | | | | | | |
| Wt/Wt | 105 | 55 | 76 | 51 | 29 | 69 | 1 | | | | | |
| Wt/*17 | 69 | 36 | 57 | 38 | 12 | 29 | 0.55 | 0.26 to 1.17 | .11 | 0.43 | 0.21 to 0.88 | .02 |
| *17/*17* | 18 | 9 | 17 | 11 | 1 | 2 | 0.15 | 0.02 to 1.21 | .04 | | | |

Abbreviations: OR, unadjusted odds ratio for genotype-frequency differences; OR_{adj} , adjusted odds ratio for carriers of variant alleles versus noncarriers, assuming a linear trend for allele dosage and adjusted for tumor size and nodal status; homEM, no null alleles (*4/*5) and not more than one *10/*41 allele; hetEM, one null allele (*4/*5) and no *10/*41 allele; IM, either two *10/*41 alleles or one null allele (*4/*5) together with one *10/*41 allele; PM, two null alleles (*4/*5); PM + IM, patients with either an IM or PM status were combined; Wt, either *1, *2, or *3 genotype.

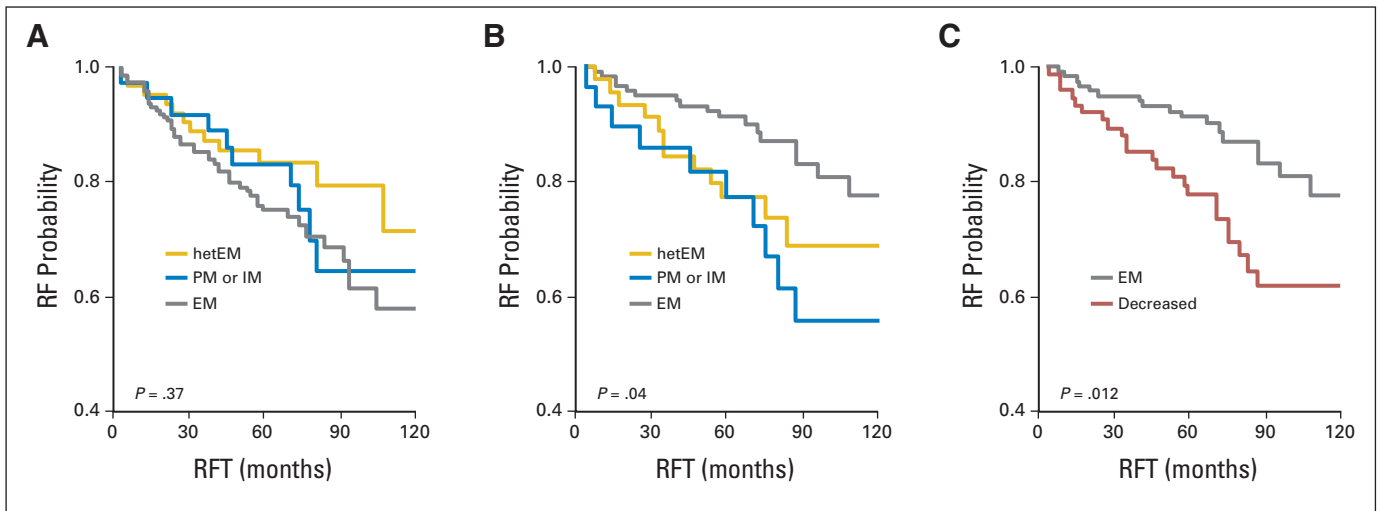


Fig 1. Kaplan-Meier probabilities of relapse-free time (RFT) for CYP2D6-metabolizer phenotypes predicted from genotypes: (A) patients not treated with tamoxifen (noTAM); (B) patients treated with adjuvant tamoxifen monotherapy (mTAM); (C) carriers of one or two impaired CYP2D6 alleles predictive for 'decreased' enzyme activity were combined. EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; hetEM, heterozygous extensive.

of risk associated with zero, one, or two impaired alleles. For the adjuvant mTAM group, Kaplan-Meier estimates show a significant, nonfavorable RFT for carriers of one or two CYP2D6 PM/IM alleles when compared with the EM genotype ($P = .04$; Fig 1B). Patients carrying at least one impaired CYP2D6 allele with an inferred decreased enzyme activity phenotype showed shorter RFTs compared with the EM phenotype ($P = .012$; Fig 1C). These differences were not observed in the control group (Fig 1A). Adjusted analysis for two prognostic factors, tumor size and nodal status, showed nonfavorable RFTs (hazard ratio [HR], 2.24; 95% CI, 1.16 to 4.33; $P = .02$) and EFS rates (HR, 1.89; 95% CI, 1.10 to 3.25; $P = .02$) in patients with decreased CYP2D6 metabolic activity (Table 3). There was a nonsig-

nificant trend that associated decreased-activity status with shorter OS (HR, 1.73; 95% CI, 0.88 to 3.41; $P = .11$).

The two CYP2C19-promoter polymorphisms that defined allele *17 (ie, $-806C > T$ and $-3402C > T$) were significantly associated with a lower risk for relapse in the mTAM patient group (OR, 0.46; 95% CI, 0.25 to 0.86; $P_{\text{emp}} = .03$) than in the control group. With respect to genotype frequencies, the risk of relapse was reduced for genotypes with one or two *17 alleles, assuming a linear trend of allele dosage (OR_{adj}, 0.43; 95% CI, 0.21 to 0.88; $P = .02$; Table 2). SNP $-806C > T$ was in strong linkage disequilibrium with SNP $-3402C > T$. The association between the CYP2C19 genotype and the clinical outcome was demonstrated by Kaplan-Meier analysis. We observed a more favorable RFT for carriers of one or two CYP2C19 *17 alleles in the mTAM group when compared with the remainder of *1, *2, and *3 carriers ($P = .05$; Fig 2B). This effect was stronger when homozygous and heterozygous *17 carriers were combined ($P = .03$; Fig 2C). No such effect was observed in the control group (Fig 2A). Cox regression showed significantly more favorable RFTs (HR, 0.45; 95% CI, 0.21 to 0.92; $P = .03$) and improved EFS rates (HR, 0.58; 95% CI, 0.32 to 1.01; $P = .05$) for carriers of a *17 genotype (Table 3). Carriers of the *17 allele tended to have a longer OS rate, although it was not a statistically significant difference (HR, 0.61; 95% CI, 0.29 to 1.26; $P = .18$).

Kaplan-Meier analyses with combined CYP2D6 and CYP2C19 genotypes show that carriers of one CYP2D6 null allele (hetEM) with a moderate to nonfavorable tamoxifen-treatment outcome can be further stratified according to their CYP2C19 genotype status as a *17 carrier or not (Fig 3). This stratification allows more accurate discrimination of patients with poor and moderate outcomes within the hetEM group. Likewise, a proportion of 32% of patients with a favorable outcome could be identified based on a nonimpaired CYP2D6 status and increased CYP2C19 activity.

No associations between genotypes and treatment outcome or survival were observed for the remaining polymorphisms of CYP2C19 (*2 and *3), CYP3A5, CYP2B6, and CYP2C9.

Table 3. Cox Proportional Hazard Ratios for CYP2D6-Metabolizer Phenotypes and for CYP2C19 *17 Genotypes in Tamoxifen-Treated Patients

| Outcome/Predictor | HR | 95% CI | <i>P</i> |
|----------------------------|------|--------------|----------|
| Relapse-free time | | | |
| CYP2D6 | | | |
| hetEM v homEM | 1.88 | 0.89 to 4.02 | .09 |
| PM or IM v homEM | 1.63 | 1.07 to 2.46 | .02 |
| Decreased v extensive | 2.24 | 1.16 to 4.33 | .02 |
| CYP2C19 | | | |
| *17 v Wt | 0.45 | 0.21 to 0.92 | .03 |
| Event-free survival | | | |
| CYP2D6 | | | |
| hetEM v homEM | 1.68 | 0.91 to 3.15 | .09 |
| PM or IM v homEM | 1.46 | 1.03 to 2.07 | .03 |
| Decreased* v extensive | 1.89 | 1.10 to 3.25 | .02 |
| CYP2C19 | | | |
| *17 v Wt | 0.58 | 0.32 to 1.01 | .05 |

Abbreviations: HR, hazard ratio adjusted for tumor size and nodal status; homEM, no null allele (*4/*5) and not more than one *10/*41 allele; hetEM, one null allele (*4/*5) and no *10/*41 allele; PM or IM, either two null alleles (*4/*5), two *10/*41 alleles, or one null allele (*4/*5) together with one *10/*41 allele; Wt, either *1, *2, or *3 genotype.

*Decreased: combined hetEMs and PM/IM.

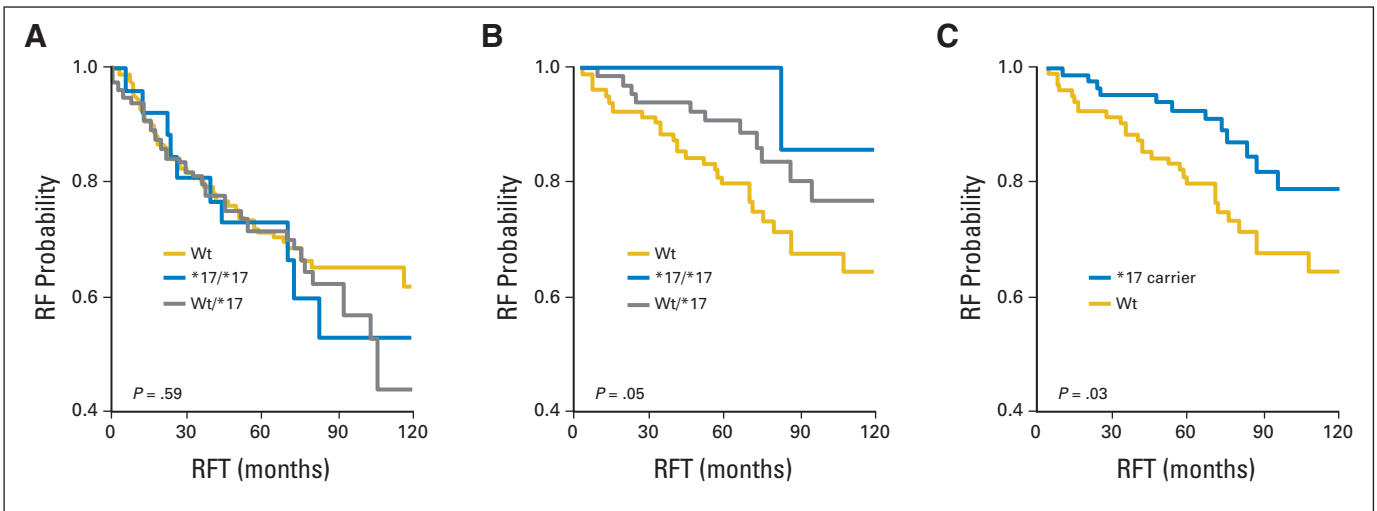


Fig 2. Kaplan-Meier probabilities of relapse-free time (RFT) for CYP2C19 genotypes: (A) patients not treated with tamoxifen (noTAM); (B) patients treated with adjuvant tamoxifen monotherapy (mTAM); (C) heterozygous and homozygous *17 allele carriers were combined; Wt, the combination of *1, *2, *3 genotypes; *17, variant allele putatively predictive for increased enzyme function.

DISCUSSION

We demonstrated a strong association between CYP2D6-PM and-IM genotypes and a nonfavorable treatment outcome in breast cancer patients receiving adjuvant tamoxifen therapy. Using the occurrence of a breast cancer event as the primary end point, our analyses support the feasibility of treatment-outcome prediction based on the patients'

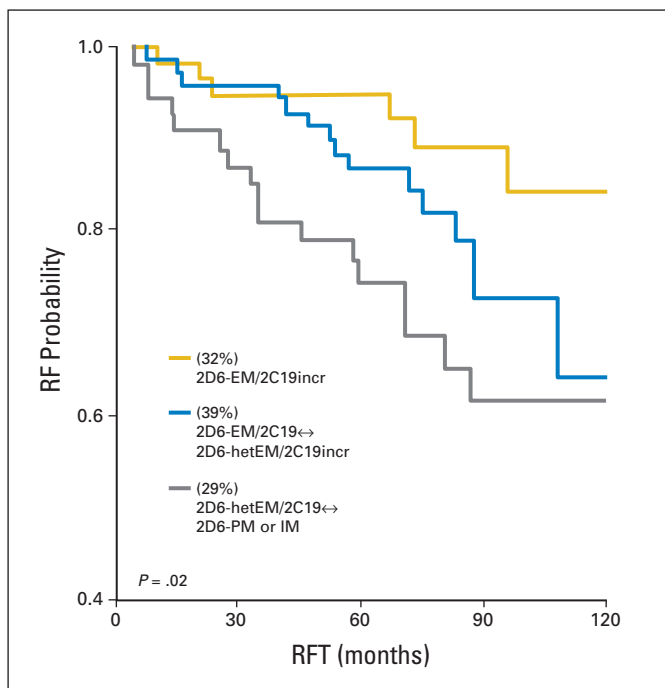


Fig 3. Kaplan-Meier probabilities of relapse-free time (RFT) of patients treated with adjuvant tamoxifen monotherapy as a function of combined CYP2D6 and CYP2C19 phenotypes inferred from genotypes. Phenotypes: CYP2D6-EM, -hetEM, -PM or IM, normal, reduced, or inactive; 2C19incr↔, nonincreased and increased activity. EM, extensive metabolizer; hetEM, heterozygous extensive; PM, poor metabolizer; IM, intermediate metabolizer.

genetic constitution. In addition to verification in PMs, this pharmacogenetic relationship has been verified for the first time for patients with CYP2D6-IM status (ie, patients homozygous for either *41 or *10 or heterozygous in combination with null alleles), through comparison with patients not treated with tamoxifen. Moreover, we provide primary genetic evidence for a CYP2C19 polymorphism as a putative supplementary biomarker for the classification of patients with a favorable treatment outcome. In the absence of any other significant genotype-outcome relationship of the tested bioactivation enzymes, although the strongest end point (OS) did not reach statistical significance because of a limited sample size, our study emphasizes CYP2D6 and CYP2C19 as tamoxifen-predictive markers. The biologic plausibility of the CYP2D6 findings is easily explained by virtue of its function to primarily catalyze the formation of the pharmacologic active, antiestrogenic metabolite, endoxifen.⁹ Our finding with CYP2C19 is novel, and its specific role within tamoxifen metabolism is less distinct.

CYP2D6 carriers of PM alleles *4 or *5 showed a greater risk for breast cancer relapse compared with patients who had normal CYP2D6 genotypes. This draws substance from our comprehensive genotyping approach that increased the detection rate of expected PMs among European patients by 10% to 15% compared with previous studies. Importantly, the non-genotyped null alleles *3, *6, *7, and *8 account for no more than 5% to 6% of overall detectable PMs,^{18,26} which corresponds to approximately one missed PM in the tamoxifen patient group. Moreover, as a unique feature, we performed combined analyses of carriers with either an IM or a homozygous-PM genotype. This allowed us to strengthen the findings of an association between RFTs or EFS rates and functionally impaired CYP2D6 variants, and it confirmed findings by Borges et al,¹⁴ who showed that IM-genotype carriers had low plasma levels of endoxifen, similar to homozygous-PM patients. We suggest that two functional CYP2D6 copies are required to receive a maximum benefit from tamoxifen treatment; this hypothesis is corroborated by our observed reduction in RFT rates in heterozygous-EM patients.

The genetic paradigm of tamoxifen-treatment outcome has been accentuated recently by clinical observations of an impact of

SSRI-comedication on the efficacy of tamoxifen treatment. Compelling pharmacologic evidence has been provided for the SSRI-associated, CYP2D6-inhibitory effects that transform the enzyme into a functionally impaired status, hence phenocopying a genetic impediment.^{14,16,17} The current study did not account for SSRI-comedication, because this information was not sufficiently available. However, some of our genotyped EM patients may have been subject to false phenotypic classification because of possible comedication with strong CYP2D6 inhibitors, such as paroxetine or fluoxetine, so our CYP2D6-PM assessment must be considered a minimum estimate.

Although our study and studies by Goetz et al^{15,16} identified the genetic CYP2D6 status as an independent predictor for the outcome of tamoxifen treatment in women with early breast cancer, others did not observe this relationship.^{21,22,23} Discrepant results may be explained by differences in study designs, including size, different genetic models for the assessment of phenotypes, or stratification effects.^{27,28} Beyond that, it is important to note that the studies of others were limited to evaluation of the CYP2D6 *4 allele and the infrequent *6 allele.¹⁵ We extended the assessment of the patient subpopulation with decreased CYP2D6-metabolizer status and predicted low endoxifen levels from 6% to 15% by conducting additional testing for the gene-deletion allele *5, and by combined IM- and PM-genotype analysis, which considerably increased the power of our study.

Our data point to an additional role of CYP2C19 *17 as a putative predictive marker, in that carriers of the CYP2C19 *17 genotype had a significant benefit trend toward reduced breast cancer recurrences and prolonged RFTs and EFS rates. The biologic plausibility for this relationship may be explained by increased gene expression of the CYP2C19 *17 allele, resulting in a putative ultra-rapid (UM) phenotype.²⁹ CYP2C19 contributes to metabolism of tamoxifen to the antiestrogenic metabolite 4-OH-TAM, and it has *in vitro* activities similar to CYP2D6.^{9,12} It also contributes to the primary oxidation of tamoxifen to *N*-desmethyltamoxifen by an estimated *in vivo* rate of 7% (unpublished data). Although the overall contribution of CYP2C19 to the formation of antiestrogenic tamoxifen metabolites is probably moderate, carriers of CYP2C19 *17 may benefit from tamoxifen administration by an increased biotransformation of active metabolites. This CYP2C19 pathway may be relevant for patients with low levels of CYP3A4 or CYP2D6. The putative clinical significance of CYP2C19 *17 becomes evident from its 24% frequency in individuals of European descent. Therefore, we suggest that CYP2C19 *17 may serve as an auxiliary biomarker for the classification of breast cancer patients likely to benefit from tamoxifen.

Our clinical association data are entirely consistent with recent findings of 4-OH-TAM and endoxifen as the antiestrogenic, active

metabolites¹¹ with similar effects on global gene expression.³⁰ Decreased or increased plasma levels of these metabolites and therefore the response to tamoxifen may be predictable, as it has become evident from our combined RFT analysis of CYP2D6/CYP2C19 genotypes. Accordingly, a maximum treatment benefit may be expected for one third of all women receiving adjuvant tamoxifen. However, other, unexplored tamoxifen metabolites with agonistic effects on ER may counteract the known antiestrogenic effects, thus pointing to a possible role of defective CYP3A5 variants.²³

Our findings are particularly important in light of the current debate on the effectiveness of tamoxifen for postmenopausal women with hormone receptor-positive breast cancer. Randomized trials demonstrated that the administration of an aromatase inhibitor after 5 years of tamoxifen³¹ or after 2 to 3 years tamoxifen^{32,33} significantly prolonged disease-free and overall survival. Because the definition of patient groups with nonfavorable and favorable tamoxifen treatment appears feasible through a priori genetic assessment of CYP2D6 and possibly of CYP2C19 metabolism, and given the long-term experience of tamoxifen as a safe and effective antihormonal treatment, we suggest using these assets to refine the choice of and/or sequencing of hormonal therapy in prospective clinical trials. In the future, the up-front, genotype-based prediction of outcome may add and refine the tailoring of antihormonal treatment strategies.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

AUTHOR CONTRIBUTIONS

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Appendix

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).