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Comprehensive CYP2D6 genotype and adherence affect outcome in breast cancer patients treated with tamoxifen monotherapy.

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Abstract

Purpose The association between *CYP2D6* genotype and outcome in breast cancer patients treated with adjuvant tamoxifen remains controversial. We assessed the influence of comprehensive versus limited *CYP2D6* genotype in the context of tamoxifen adherence and co-medication in a large cohort of 618 patients.

Methods Genotyping of 33 *CYP2D6* alleles, using two archival cohorts from tamoxifen-treated women with invasive breast cancer (Dundee, n=391; Manchester, n=227). Estimates for recurrence-free survival (RFS) were calculated based on inferred *CYP2D6* phenotypes using Kaplan-Meier and Cox proportional hazard models, adjusted for nodal status and tumor size.

Results Patients with at least one reduced-function *CYP2D6* allele (60%) or no functional alleles (6%) had a non-significant trend for worse RFS: hazard ratio (HR) 1.52 [CI 0.98 – 2.36, p = 0.06]. For post-menopausal women on tamoxifen monotherapy, the HR for recurrence in patients with reduced functional alleles was 1.96 [CI 1.05-3.66, p = 0.036]. However, RFS analysis limited to four common *CYP2D6* allelic variants, was no longer significant (p=0.39). The effect of *CYP2D6* genotype was increased by adjusting for adherence to tamoxifen therapy, but not significantly changed when adjusted for co-administration of potent inhibitors of *CYP2D6*.

Conclusions Comprehensive genotyping of *CYP2D6* and adherence to tamoxifen therapy may be useful to identify breast cancer patients most likely to benefit from adjuvant tamoxifen.

Keywords: adherence, cytochrome P450, CYP2D6, pharmacogenetics, tamoxifen

Abbreviations

CYP450: cytochrome p450

RFS: recurrence-free survival

HR: hazard ratio

CI: confidence intervals

ER: oestrogen receptor

PM: poor metaboliser

IM: intermediate metaboliser

EM: extensive metaboliser

UM: ultrametaboliser

HWE: Hardy-Weinberg Equilibrium

DCIS: ductal carcinoma in situ

DNA: deoxyribonucleic acid

Introduction

Despite the advent of aromatase inhibitors, tamoxifen remains an important drug in the endocrine treatment of patients with oestrogen receptor (ER) positive breast cancer [1]. Tamoxifen is a pro-drug metabolised to potent anti-oestrogenic metabolites 4-hydroxy-N-desmethyl tamoxifen (endoxifen) [2] and 4-hydroxy tamoxifen [3], predominantly by the enzyme CYP2D6. Genetic variants of *CYP2D6* can result in a range of enzyme activities from ultra-rapid to absent function with approximately 7% of Caucasian women homozygous for non-functional *CYP2D6* alleles [4].

The effectiveness of tamoxifen may be influenced by factors including CYP2D6 metabolizer genotype [5], adherence to treatment [6], co-medications which may inhibit the conversion of tamoxifen to active metabolites including endoxifen [7], and other mechanisms of molecular resistance [8].

Some studies have suggested that women with one or two non-functional variants of *CYP2D6* have a worse clinical outcome compared to women with normal CYP2D6 activity, when treated with tamoxifen in the adjuvant setting [9-13]. Others have failed to confirm an association [14], or controversially, suggested women with non-functional *CYP2D6* alleles receiving tamoxifen have a better outcome [15,16]. Such conflicting evidence may reflect the relatively small study sizes, disparate patient populations and the range of *CYP2D6* alleles determined [5]. A number of recent reviews have stated that routine testing of *CYP2D6* genotype is still not yet established in the evaluation of women with ER positive breast cancer [1,17-19].

Adherence to tamoxifen may be poor and declines over time [6], influenced by side effects from the drug [20]. Furthermore, poor adherence to adjuvant tamoxifen for breast cancer has been associated with worse survival [6]. However, no previous studies of the association between *CYP2D6* genotype and breast cancer outcome have examined this crucial covariate.

A number of co-medications are potent CYP2D6 inhibitors, including antidepressant drugs such as paroxetine and fluoxetine [7], which have been prescribed to treat hot flushes associated with tamoxifen use [21]. Adjustment for CYP2D6 inhibitors in some reports has highlighted the significance of combining genetic and environmental contributors to tamoxifen response [22,23], but data is inconsistent in establishing the influence of potent CYP2D6 inhibitors on tamoxifen response in general [24,25,26]. Indeed, recent evidence suggests that only concomitant use of paroxetine with tamoxifen is associated with increased breast cancer specific mortality [25].

We sought to determine the effect of the extent of *CYP2D6* genotyping, adherence to tamoxifen and co-administration of CYP2D6 inhibitors on breast cancer recurrence in patients treated with adjuvant tamoxifen.

Patients and Methods

Patients

CYP2D6 genotyping was performed on samples from 618 women with ER-positive breast cancer who were prescribed 20mg tamoxifen daily, for an intended five years, as adjuvant therapy. Subjects were from two geographically distinct cohorts (Figure 1).

Cohort 1 comprised 391 Caucasian women with stage I, II or III breast cancer from Dundee, UK who had frozen primary breast tissue collected prospectively between 1997 and 2007 and updated clinical and pathological data. Matched peripheral blood lymphocyte DNA was available for 133 patients; 228 women were postmenopausal and had received adjuvant tamoxifen monotherapy. Ethical approval was obtained from the Tayside Tissue Bank Local Research Ethics Committee.

For adherence data, conducted under Caldicott Guardian approval, the Community Health Index number allowed linkage of health-related datasets providing a unique resource combining information on dispensed prescribing with detailed clinical data at the individual patient level [6]. Tamoxifen adherence was calculated as previously described as cumulative exposure and patients with an adherence index less than 80% were deemed to have “low adherence” [6].

Complete co-medication data was also recorded in this cohort and focused on the use of potent *CYP2D6* inhibitors including fluoxetine, paroxetine, quinidine and bupropion (medicine.iupui.edu/clinpharm/COBRA/TamoxifenGuide.pdf).

Cohort 2 was composed of 227 Caucasian women with stage I, II or III breast cancer from Manchester, UK who had frozen primary breast tissue collected

prospectively between 1989 and 1998. 180 of these women were postmenopausal and had received adjuvant tamoxifen monotherapy. Clinical data was collected by a comprehensive retrospective case note review to supplement prospectively collected data. The study was approved by the Trafford and Salford Research and University of Manchester Ethics Committees.

Clinical data was blinded to the laboratory investigators and data sets merged only on completion of the study.

DNA Isolation and *CYP2D6* genotyping

Breast tissue from Dundee was macro-dissected immediately post-operatively and tumor was snap frozen in liquid nitrogen before storage at -80°C. Total genomic DNA was isolated using MagAttract® DNA Mini M48 Kit (Qiagen Ltd, Crawley, West Sussex, UK) on a BioRobot M48 according to the manufacturer's protocol. Tumor specimens from Manchester patients were snap frozen in liquid nitrogen and preserved in OCT medium at -80°C. DNA was extracted by the EZ1 Qiagen robot according to manufacturer's protocol. Due to limited published data on the concordance of genotyping results between blood and tumor tissue [11], we undertook *CYP2D6* genotyping in matched samples of tumor tissue and peripheral blood lymphocytes from 133 cases in cohort 1.

CYP2D6 genotyping was performed using the AmpliChip CYP450 Test (Roche Molecular Systems, Inc., Pleasanton, CA). The AmpliChip CYP450 Test queries 29 *CYP2D6* polymorphisms to identify 33 different alleles, including a variety of gene duplications. Each allele can be assigned to one of four phenotypic categories according to its associated enzyme function: poor

metabolizer (PM), intermediate metabolizer (IM), extensive metabolizer (EM) and ultra-rapid metabolizer (UM).

The predicted CYP2D6 phenotype was deduced for individual *CYP2D6* alleles (<http://www.cypalleles.ki.se>) and *CYP2D6* genotypes were classified into seven phenotypic categories using the multiple alleles, ranked from the lowest to the highest level of enzymatic function: low, PM/PM; intermediate, PM/IM, PM/EM, IM/IM, IM/EM; and high, EM/EM and EM/UM.

Statistical analysis

The association between *CYP2D6* genotype and breast cancer outcomes was assessed by collapsing the seven phenotype groups described above into three functional categories: Extensive/ultra-rapid (EM/EM and EM/UM), intermediate (EM/IM, EM/PM, IM/IM and IM/PM) and poor (PM/PM) metabolism to allow comparison with recent key publications [11, 27].

Allele frequencies were calculated and tests for Hardy-Weinberg Equilibrium (HWE) were performed (<http://www.r-project.org/>). Genotyping analysis with limited allele coverage was based on the presence of *CYP2D6* *4, *5, *10 and *41 alleles, with all other alleles detected by the AmpliChip genotyping system reassigned to the wild type allele [10].

Patients were classified by age, menopausal status, concomitant use of adjuvant chemotherapy, node status and tumor size. For those patients missing menopausal data, menopausal status was inferred from the patient's age at diagnosis: <45 years at diagnosis included in the pre-menopausal category; >55 years included in the post-menopausal category. Patients aged 45-55 years were assigned to the missing data category. Based on this

process, the status of two and 31 patients were assigned to the pre- and post-menopausal groups, respectively.

The primary planned analysis was a comparison of the hazard rate of the PM/PM group to the EM/EM group using a multivariate Cox proportional hazards models adjusted for tumor size and nodal status with relapse free survival (RFS) as the outcome. Relapse was defined as locoregional recurrence, ductal carcinoma in situ (DCIS), distant metastases, contralateral DCIS or death due to breast cancer. A secondary analysis to estimate the difference in hazard among the decreased and extensive metabolizer groups for all patients and for the postmenopausal, tamoxifen monotherapy subgroup was also planned and performed. A set of exploratory analyses to look at subgroups that were of small size but that might give indications of interest for indicating directions for future studies, were additionally planned, such as those performed to determine the effect of adjusting for adherence and comedication with the data available with relapse free survival (RFS) as the outcome. Estimates of hazard ratios for the combined patient groups were obtained by using a fixed-effects meta-analysis approach, where individual HR were calculated for each centre, and then combined as a mean, weighted by the inverse of the individual study variances.

The analyses were adjusted on the clinical covariates of nodal status and tumor size. These covariates were chosen based on associations seen in previous related studies [23]. Nodal status was classified as 0, 1-3 or 4+ nodes, and tumor size as <2cm, or ≥ 2 cm in tumor size. Because the proportional hazards assumption was violated for the patients with follow-up data of >12 years and because of the small number of patients with this length

of follow-up, the survival analysis was performed only on the data from the first 12 years of follow time which did not grossly violate the proportional hazards assumption based on log-log plots and goodness-of-fit tests of this assumption (data not shown). All analyses were performed in R (<http://www.r-project.org/>), and included the use of the package “survival”.

Results

Characteristics of the two cohorts

Clinical characteristics were similar between the two cohorts including mean age of disease diagnosis (60.5 versus 63.1 years), nodal status (node negative 52% versus 50%) tumor size <2cm (33% versus 33%). However, a greater percentage of patients in Cohort 1 had undergone chemotherapy (27% vs 4%), more patients in Cohort 1 were pre-menopausal (27% vs 5%) and Cohort 2 had a longer median follow up (9.4 versus 4.9 years).

When data from the two cohorts was analysed independently, there were no significant differences in *CYP2D6* allele frequency or outcome (Supplementary Table 1) and thus the two cohorts were combined for the analyses (Table 1). The AmpliChip CYP450 genotype call rate was 95.448% (671/703 samples). From 671 genotyped samples, 53 results were excluded due to incomplete clinical data, leaving 618 patient results for statistical analyses. The observed allele frequencies at the *CYP2D6* locus are listed in Supplementary Table 2. Overall 60% of patients had at least one reduced-function *CYP2D6* allele and 6% had no functional *CYP2D6* alleles. Sensitivity analysis indicated that combining the two sets of data did not alter the inferences made from the Cox Proportional Hazards model. There were 137 recurrence events among the 618 (22.2%) patients during the follow up period.

Concordance between tumor and germline genotype

Paired samples of tumor DNA and lymphocyte DNA were available from 133 patients in cohort 1. The *CYP2D6* genotype concordance was 100% (data not

shown) indicating that *CYP2D6* genotype determination from tumor tissue accurately reflects the patients' germline genotype.

Recurrence-free survival analyses according to *CYP2D6* metabolism groups

As part of our pre-planned statistical analysis, we compared patients who were homozygous for PM *CYP2D6* alleles with patients who had at least two normally functioning *CYP2D6* alleles (EM). Somewhat surprisingly, we observed no breast cancer recurrences in the 27 patients comprising the PM/PM group for whom complete covariate data was available. This lack of events in this small PM/PM group may be attributed to the higher prevalence of favourable clinical characteristics including tumors <2cm (59% versus 34%, $p = 0.01$), less node-positive disease (37% vs 45%), and fewer pre-menopausal women (15% vs. 21%) (Supplementary Table 3).

Because of their surprising nature, it was appropriate to perform a *post hoc* power analysis of these results. For this data set, there was approximately 55% power to detect a hazard ratio of 1.5 in the comparison among PM/PM to the extensive metabolizer patients. Therefore, it is possible that the PM/PM group may have a greater risk of recurrence than the EM/EM group, but this study was unable to definitively test that hypothesis. Although the study was underpowered to detect anything other than a large effect of *CYP2D6* on recurrence rate when this analysis was planned, because of some large effects that had been reported in the literature and because of the relevance to the rest of the planned analyses, this comparison was performed.

Another pre-planned statistical analysis compared the outcome between patients with predicted normal *CYP2D6* activity (UM/ EM and EM/EM

patients) to patients with any reduced function alleles. This analysis, despite the lack of events in the PM group, demonstrated a reduced RFS in patients with one or more reduced function or null alleles (HR 1.52, 95%CI 0.98-2.36, p=0.06), with adjustment for covariates (Figure 2, Table 2).

Sub-group analysis considering only post-menopausal women who received adjuvant tamoxifen as monotherapy demonstrated that decreased metabolizers (including all patients with one or more reduced function or null alleles) had a significantly greater relative risk of breast cancer recurrence (HR 1.96, 95%CI 1.05-3.66, p=0.04) (Table 2) both with and without covariate adjustment for tumor size and nodal status (Figure 3).

Comparison of the association between comprehensive versus limited *CYP2D6* genotyping and RFS

Previous assessments of the relationship between *CYP2D6* genotype and response to tamoxifen treatment in patients with breast cancer have considered a limited panel of *CYP2D6* variant alleles, either *CYP2D6**4 alone [22,28] or a selected panel of four more common variant alleles - *CYP2D6**4, *5, *10 and *41 [10,23]. We observed *in silico* that re-assigning *CYP2D6* genotype to a limited panel of reduced/null function alleles (*CYP2D6**4, *5, *10 and *41) compared with those classified as having normal *CYP2D6* function resulted in no significant differences (Table 2). This analysis indicates that limited *CYP2D6* allele coverage may result in the misclassification of some patients with reduced *CYP2D6* function as extensive metabolizers in keeping with recent evidence [27].

Influence of tamoxifen adherence on RFS

Tamoxifen adherence data was available on 257 patients in cohort 1, based on linkage of their complete records of prescription encashment in the community. Thirty seven patients had adherence less than 80%, a level previously associated with an increased risk of breast cancer recurrence [6]. Two of these 37 patients had recurrence events and were extensive metabolizers. Reassigning patients in cohort 1 with adherence <80% to the decreased metabolizer group (Figure 4) resulted in the hazard ratio of this group increasing from the multivariate HR of 2.57 ($p=0.03$) to a HR of 3.02 (1.07, 8.47), $p=0.04$. Within the postmenopausal subset treated with tamoxifen only, reassigning patients based on adherence showed a change from initial multivariate HR of 7.14 ($p=0.06$) to a HR of 5.57 (0.74, 41.77), $p=0.09$. After adjusting for adherence, patients within the extensive metabolizer group had an extremely low breast cancer recurrence rate.

Influence of CYP2D6 inhibitors on RFS

The CYP2D6 metabolism status of patients was adjusted for co-medication by re-classifying those patients in the extensive metabolizer group (UM+EM) who were prescribed either fluoxetine or paroxetine, both strong inhibitors of CYP2D6, as decreased metabolizers (no patients were taking quinidine or bupropion). In the 424 patients with either available co-medication data and/or genetically decreased metabolism status, 32 (7.6%) were known to be prescribed a strong inhibitor. Of these, 14 EM patients were reclassified to decreased metabolizers based on inhibitory co-medications, and adjustment for these co-medications did not alter the HR (data not shown).

Discussion

There is growing interest in personalized medicine and in the use of genetic testing to provide information to select specific treatments for patients with cancer. The influence of *CYP2D6* polymorphisms on outcome in tamoxifen-treated, ER-positive breast cancer patients remains controversial [9-19]. The current study was conducted in routine practice and supports the association between *CYP2D6* polymorphisms and clinical outcome [9-14,22,23], but highlights the importance of comprehensive genotyping and particularly the adverse effect of poor adherence [6,26,29,30].

The failure to observe an increased recurrence risk in breast cancer patients with a poor metabolizer (PM) phenotype was surprising. The lack of events in this group may be attributed to the small number of patients and, by chance, the more favourable clinical features (Supplementary Table 3) compared to the other genotype groups or simply due to random sampling effects.

Nonetheless, we observed an increased breast cancer risk amongst the much larger group of patients with decreased metabolism based on the presence of one or more hypofunctional *CYP2D6* alleles. Hypofunctional alleles in our analysis included both intermediate and null alleles. By these criteria, 60% of the treated women had one or more hypofunctional alleles compared to only 6% who had two null alleles and would be predicted to poor metabolizers.

However, it is important to note that different conclusions may be drawn from the data if the groups for comparison were defined differently and further work is required to establish the most appropriate classification of *CYP2D6* status in relation to tamoxifen. The detrimental effect of carrying one or more hypofunctional alleles was most apparent in the post-menopausal cohort

treated with tamoxifen monotherapy, who received no adjuvant cytotoxic chemotherapy. The separation of the survival curves for extensive and reduce metabolizers (Figure 3) occurred earlier (~2.5 years) in the post-menopausal tamoxifen monotherapy group than in the entire cohort (~ 4 years). This mirrors the findings of Goetz et al [22] and further confirms that the effect of *CYP2D6* metabolizer status is greater in this post-menopausal patient subset. Although prior studies established the adverse effect of a *CYP2D6* poor metabolizer genotype on outcome [22,23], other studies have demonstrated that individuals with one or more hypofunctional alleles have an increased risk of breast cancer recurrence when treated with tamoxifen [10,11]. Together these data suggest that only patients with two fully functional *CYP2D6* alleles experience the full clinical benefit of tamoxifen. Many previous studies of *CYP2D6* genotyping and tamoxifen have used formalin fixed paraffin embedded tissue [10,22] and genotype assays, which define a limited number of alleles. The availability here of DNA from fresh-frozen tumor tissue permitted genotyping for a wider range of variant *CYP2D6* alleles. The correlation between tumor and blood DNA provides justification for the use of frozen tumor DNA as source material for *CYP2D6* genotyping if blood samples are not available.

The association between *CYP2D6* genotype and outcome in our cohort was statistically significant only with the extended *CYP2D6* allele coverage. This suggests that the method of genotyping may be critical for accurate phenotype prediction [17] and the results of prior studies of *CYP2D6* genotype may have been confounded by the inadvertent mis-assignment of some patients to a normal or extensive metabolizer phenotype. This is consistent

with previous reports that highlight the importance of broad *CYP2D6* variant allele coverage [27,31], which is critical to identify hypofunctional alleles present in certain ethnic minorities [4].

Reliable adherence data is rarely available outside the context of clinical trials [30], but was uniquely available in this study for a proportion of patients through clinical record linkage [6]. Women who are *CYP2D6* extensive metabolizers are more likely to have poor adherence and to discontinue tamoxifen as they experience more severe side effects related to oestrogen deprivation [20]. Thus, those patients who are most likely to benefit from tamoxifen therapy paradoxically may also have the highest incidence of side effects. While symptomatic data or tamoxifen metabolite levels were not available in the present study and the reasons for reduced adherence remain uncertain, whatever the cause, poor adherence enhanced the effect of reduced *CYP2D6* function, emphasizing the importance of adherence on disease free and overall survival from breast cancer [6,26,29,30].

While *CYP2D6* inhibitors reduce endoxifen levels [13,21] and have been associated with worse outcome in patients treated with tamoxifen [22,23,25], there was no significant adverse effect of potent *CYP2D6* inhibitors in this study. This may reflect the infrequent use of such medications in the two cohorts, as only a small number of patients were identified as having taken potent inhibitors. Recent evidence suggests that amongst antidepressants, also used for reducing side effects from tamoxifen, only paroxetine has a significant effect on breast cancer survival [25]. Further studies considering the effects of *CYP2D6* inhibitors are required to establish the relevance of this potential drug interaction on clinical outcome.

Conclusions

The decision whether to adopt CYP2D6 testing into routine clinical practice will require analysis of large randomised controlled trial datasets and large meta-analyses such as those being conducted by the international tamoxifen pharmacogenetics consortium. However, for adjuvant therapy after ER positive breast cancer, we recommend that comprehensive genotyping should be considered and this may utilise either constitutional or tumor DNA.

Furthermore, the *CYP2D6* genotype needs to be interpreted in the context of the clinical setting where adherence to prescribed tamoxifen therapy may have a significant influence on disease recurrence.

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Declaration of competing interests

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Figure Legends

Figure 1. Flow diagram of patient cohorts

Figure 2. Unadjusted Kaplan-Meier curves for all patients with complete covariate data, combined across centers, by CYP2D6 metabolism groups

Figure 3. Unadjusted Kaplan-Meier curves for tamoxifen monotherapy, post-menopausal patients by CYP2D6 metabolism groups

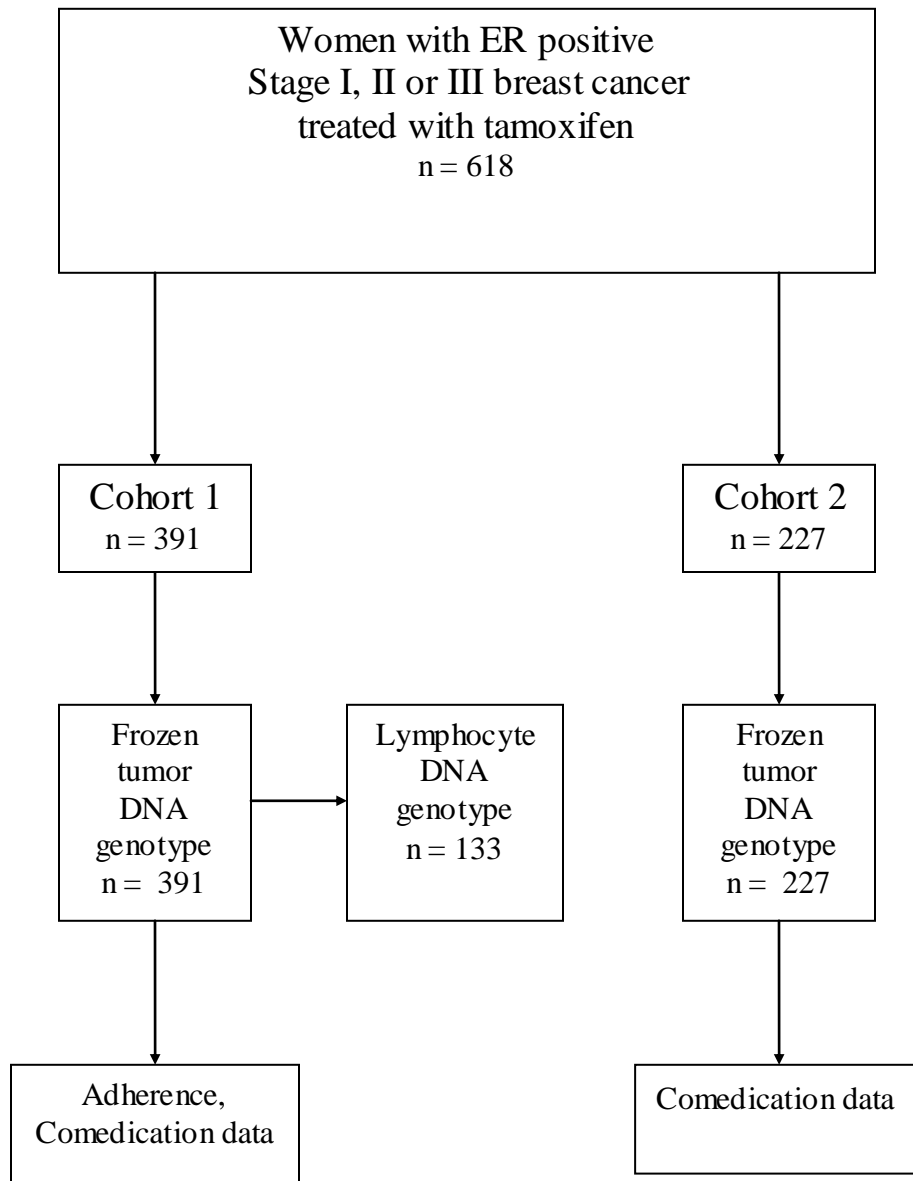
Figure 4. KM-curves of genotypes adjusted for adherence

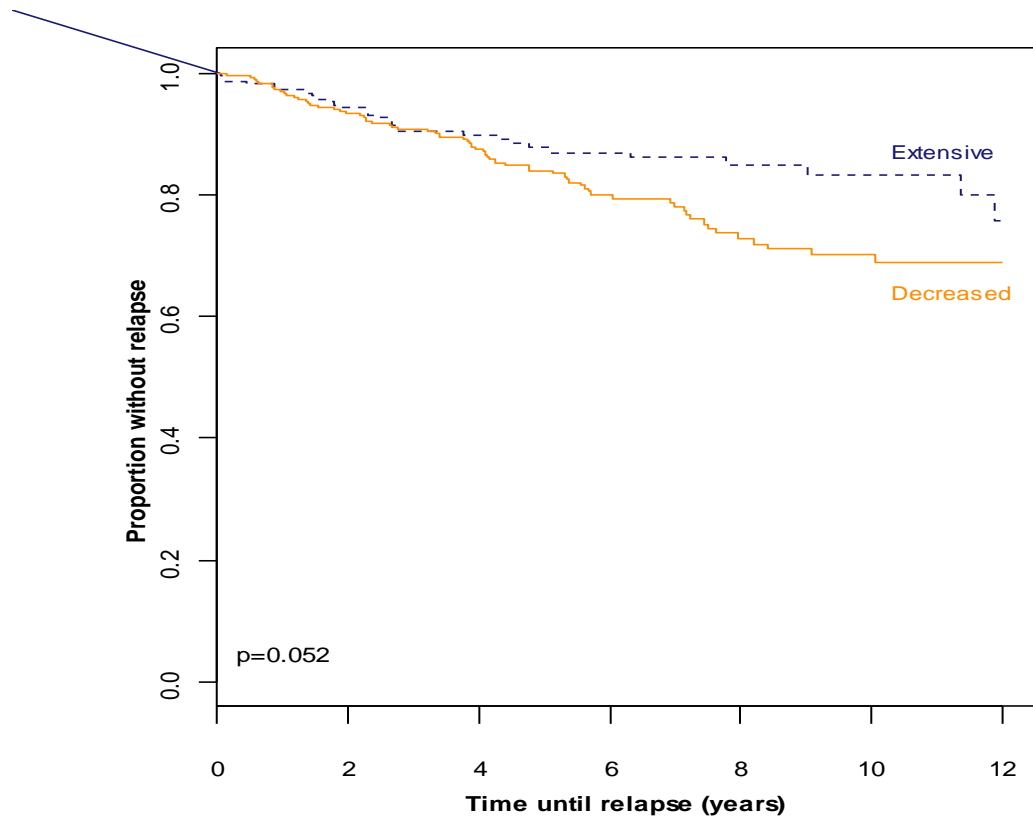
Table 1: Clinical characteristics of the entire patient cohort, based on *CYP2D6* genotype groupings.

	<i>CYP2D6</i> Genotype Group						
	PM/PM	IM/PM	IM/IM	EM/PM	EM/IM	EM/EM	UM/EM
Num. Patients	34	31	13	171	126	234	9
Percent Recurred	12	29	0	22	31	15	33
Age (Years)							
Mean	63.4	63.8	64.7	61.1	62.4	60.5	59.8
Std. Dev	13.3	14.1	12.9	13.4	13.2	13.0	22.4
Menopausal Status (%)							
Pre	4	5	1	36	22	48	33
Post	26	25	11	129	97	169	67
Missing	4	1	1	6	7	17	0
Median Follow Time (Years)	6.8	4.0	4.1	5.9	4.3	6.1	4.7
Chemotherapy (%)	15	19	8	18	22	18	22
Nodal Status (%)							
0 nodes	50	42	85	54	48	50	67
1-3 nodes	32	39	0	31	25	35	11
4+ nodes	12	16	8	12	24	12	22
Missing	6	3	8	4	4	3	0
Tumor Size (%)							
<2cm	50	32	31	39	21	32	22
2-5cm	29	61	46	51	63	53	44
>5cm	6	0	8	4	6	4	22
Missing	15	6	15	6	10	11	11

Table 2: Estimates of hazard ratios (HR) of the risk of relapse of decreased patients relative to the risk of extensive metabolizers for two types of genotyping and effect estimates for the covariates in the multivariate models. Comprehensive genotyping detects the presence of 33 *CYP2D6* alleles, while limited genotyping detects the presence of *CYP2D6**4, *5, *10, and *41. All other alleles detected by the comprehensive genotyping would be treated as wild type with the limited genotyping. HRs were adjusted for tumor size and nodal status.

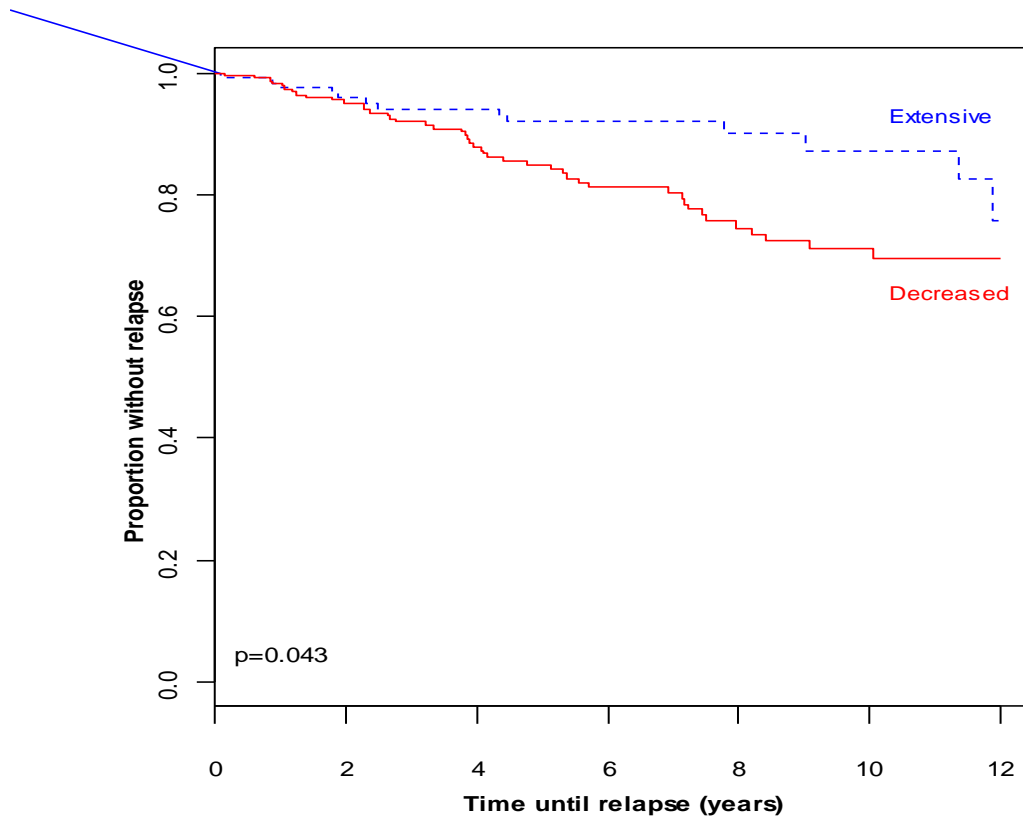
Group	Variable	HR	95% CI	p-value
All Patients	CYP2D6 Comprehensive Genotyping	1.52	(0.98, 2.36)	0.06
	CYP2D6 Limited Genotyping	1.03	(0.67, 1.58)	0.88
	Tumor Size (≥ 2cm vs. < 2cm)	1.36	(0.84, 2.19)	0.21
	Nodal Status (1-3 nodes vs. 0 nodes)	1.61	(0.95, 2.73)	0.08
	Nodal Status (4+ nodes vs. 0 nodes)	4.81	(2.91, 7.96)	9.06 x 10 ⁻¹⁰
Postmenopausal, Tamoxifen Monotherapy	CYP2D6 Comprehensive Genotyping	1.96	(1.05, 3.66)	0.04
	CYP2D6 Limited Genotyping	1.26	(0.74, 2.16)	0.39
	Tumor Size (≥ 2cm vs. <2cm)	1.38	(0.77, 2.49)	0.28
	Nodal Status (1-3 nodes vs. 0 nodes)	1.43	(0.70, 2.92)	0.33
	Nodal Status (4+ nodes vs. 0 nodes)	5.32	(2.88, 9.81)	9.01 x 10 ⁻⁸





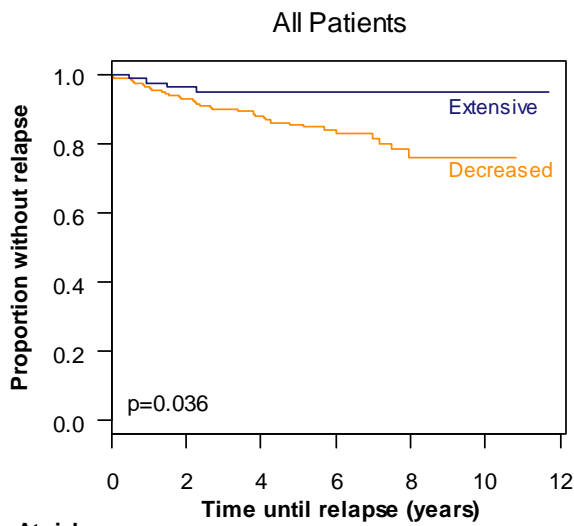
Patients at risk

Extensive	213	184	137	103	65	39	18
Decreased	329	280	206	138	88	53	23



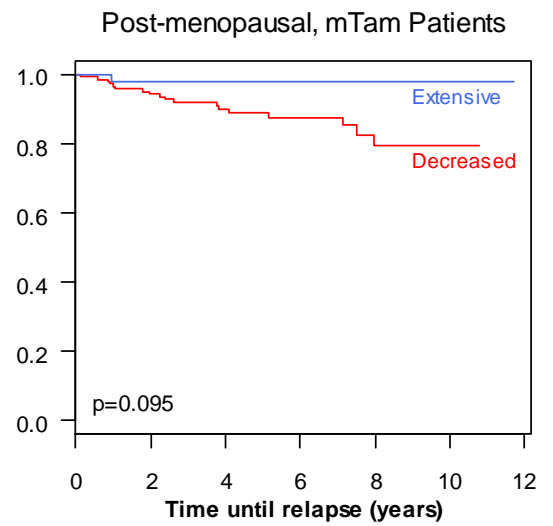
Patients at risk

Extensive	126	112	89	69	42	27	11
Decreased	225	195	145	100	71	47	18



At risk

Ext	85	73	41	27	6	2	1
Decr	243	202	139	81	36	7	0



Ext	48	44	26	19	3	1	1
Decr	148	124	86	50	25	5	0