Predictive Biomarkers and Personalized Medicine

Clinical Cancer Research

CYP2D6 Metabolism and Patient Outcome in the Austrian Breast and Colorectal Cancer Study Group Trial (ABCSG) 8

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Abstract

Purpose: Controversy exists about *CYP2D6* genotype and tamoxifen efficacy.

Experimental Design: A matched case–control study was conducted using the Austrian Breast and Colorectal Cancer Study Group Trial 8 (ABCSG8) that randomized postmenopausal women with estrogen receptor (ER)-positive breast cancer to tamoxifen for 5 years (arm A) or tamoxifen for 2 years followed by anastrozole for 3 years (arm B). Cases had disease recurrence, contralateral breast cancer, second non–breast cancer, or died. For each case, controls were identified from the same treatment arm of similar age, surgery/radiation, and tumor–node—metastasis (TNM) stage. Genotyping was conducted for alleles associated with no (PM; *3, *4, *6), reduced (IM; *10, and *41), and extensive (EM: absence of these alleles) CYP2D6 metabolism.

Results: The common *CYP2D6*4* allele was in Hardy–Weinberg equilibrium. In arm A during the first 5 years of therapy, women with two poor alleles [PM/PM: OR, 2.45; 95% confidence interval (CI), 1.05–5.73, $P = 0.04$] and women with one poor allele (PM/IM or PM/EM: OR, 1.67; 95% CI, 0.95–2.93; $P = 0.07$) had a higher likelihood of an event than women with two extensive alleles (EM/EM). In years 3 to 5 when patients remained on tamoxifen (arm A) or switched to anastrozole (arm B), PM/PM tended toward a higher likelihood of a disease event relative to EM/EM (OR, 2.40; 95% CI, 0.86–6.66; $P = 0.09$) among women on arm A but not among women on arm B (OR, 0.28; 95% CI, 0.03–2.30).

Conclusion: In ABCSG8, the negative effects of reduced CYP2D6 metabolism were observed only during the period of tamoxifen administration and not after switching to anastrozole. *Clin Cancer Res; 19(2); 500–7. ©2012 AACR.*

Introduction

In the adjuvant treatment of postmenopausal estrogen receptor (ER)-positive breast cancer, 5 years of an aromatase inhibitor (AI) or a sequencing regimen of 2 years of tamoxifen followed by 3 years of an aromatase inhibitor significantly prolongs disease-free survival (DFS) compared with 5 years of tamoxifen (1). On the basis of these data, practice guidelines recommend either an aromatase inhibitor for 5 years or the sequence of tamoxifen followed by an aromatase inhibitor (2).

Tamoxifen is a weak anti-estrogen with agonistic properties that is extensively metabolized into potent anti-estrogens, 4-hydroxy tamoxifen, and endoxifen, which exhibit similar potency in terms of binding affinity to ERs (3), suppression of estradiol-stimulated cell proliferation (4), and gene expression (3). Endoxifen is formed by the CYP2D6-mediated oxidation of *n*-desmethyl tamoxifen (4, 5). Common genetic variations in CYP2D6 and/or drug-induced inhibition of CYP2D6 enzyme activity are associated with significant reductions in endoxifen concentrations in tamoxifen-treated humans (6–8). These data led to the hypothesis that CYP2D6 variation may affect the clinical outcomes of women treated with tamoxifen but not anastrozole, as CYP3A and not CYP2D6 is the major P450 isoform involved in the metabolism of anastrozole (9).

There has been great heterogeneity with regard to the reported association between CYP2D6 metabolism and clinical outcomes. Retrospective data from a randomized clinical trial of women treated with tamoxifen monotherapy for early-stage ER-positive breast cancer (NCCTG 89-30-52; ref. 10), a pooled analysis of the women from NCCTG 89-30-52 and a respective German cohort (11) and other reports (reviewed in ref. 12) have shown an association

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Translational Relevance

There is controversy whether the efficacy of tamoxifen is altered in women with genetic or drug-induced alterations in CYP2D6, the rate-limiting enzyme responsible for the metabolism of tamoxifen to its active metabolite, endoxifen. Whereas many negative studies have evaluated CYP2D6 polymorphisms in patients with a history of tamoxifen use, data from Austrian Breast and Colorectal Cancer Study Group Trial 8 (ABSCG8) show that variation in CYP2D6 metabolism is associated with a higher risk of recurrence only during the period of tamoxifen administration and not after switching to anastrozole. These data suggest that future studies should prospectively evaluate novel strategies to overcome the limitations of CYP2D6 metabolism, including the direct administration of endoxifen.

between *CYP2D6* genotype and DFS. However, multiple other reports have not shown an association (reviewed in ref. 12) including a recent analyses of a subset of patients enrolled in 2 prospective adjuvant clinical trials, ATAC and BIG 1-98 (13, 14). However, concern has been raised (15) given the observation of substantial departure from Hardy–Weinberg equilibrium (HWE) for the most important *CYP2D6* allele, **4* in both studies (13, 14) Moreover, a review evaluating published studies found large between-study variability in the classification of genotypes and choice of primary endpoint as well as substantial methodologic shortcomings leading to inconsistencies in study conclusions (16). Therefore, we sought to obtain independent determination whether the odds of a disease event differs by *CYP2D6* genotype in women with early-stage ER-positive breast cancer who received 5 years of tamoxifen as adjuvant therapy by conducting a secondary analysis of a large prospective tamoxifen study. We also aimed to determine the relationship between *CYP2D6* genotype and outcomes in women receiving 2 years of tamoxifen followed by 3 years of anastrozole.

Methods

Patients

The source of patients was the Austrian Breast and Colorectal Study 8 (ABCSG trial 8, NCT00291759), a prospective, multicenter, randomized, open-label trial that randomized 3,901 surgically resected patients with early-stage breast cancer within 6 weeks after surgery to either 5 years of tamoxifen (20 mg/daily) or to 2 years of tamoxifen (20 mg/daily) followed by a switch to anastrozole (1 mg/d) for 3 years (17). Eligible patients were postmenopausal women aged 80 years or younger with histologically verified ductal or lobular breast carcinoma that was invasive or minimally invasive, endocrine-responsive, and Nottingham grade 1 or grade 2. Neoadjuvant chemotherapy (CT), hormone therapy (HT), or radiotherapy (RT) was not allowed. Patients underwent modified radical mastectomy or breast-conserving surgery with axillary lymph node dissection or sentinel lymph node biopsy (with or without subsequent RT). None of the patients received adjuvant CT. All patients provided written informed consent in accordance with the Declaration of Helsinki. The pharmacogenetics substudy was approved by the relevant ethics committees in Austria and the United States.

Sample preparation

To overcome the potential problems related to somatic deletion of the CYP2D6 chromosomal locus on 22q13 (18, 19), 3 unmounted whole-tissue sections (10-μm thick) derived from paraffin-embedded tissue blocks containing both normal and tumor tissue were prepared. One hematoxylin and eosin slide was also obtained to confirm tissue cellularity. From the unmounted whole sections, the tissue was deparaffinized, and DNA extracted using the modified method of Schroth and colleagues (11).

Assay methods

DNA was assessed for the most common CYP2D6 single-nucleotide polymorphisms (SNP) corresponding to alleles associated with null [*3 (2549 del A),*4(1846G>A), and *6 (1707T>del)] reduced [*10 (100 C>T and 1846 G>A) and *41 (2988 G>A)] CYP2D6 enzyme activity as previously described (11) using the Applied Biosystems Taqman Allelic Discrimination Assay with the ABI Prism 7900HT Real Time System according to the manufacturer's instructions. To ensure that nonspecific amplification was not misinterpreted, the amplification plots were evaluated in addition to the endpoint allelic discrimination plots. Two of the 3 triplicate reactions must concur and data derived from amplification beyond 45 cycles were not used. Samples from the Coriell Institute (Camden, NJ) with known genotypes for each SNP and each possible allele combination (when available) were included on every plate and evaluated along with the unknown genotype samples. The presence of the alleles in the Coriell samples were confirmed by sequencing with validated methods that meet HWE standards. The real-time methods were validated against the previously validated PCR and sequencing methods. A pooled DNA sample from ABI was also used in a standard curve to estimate the level of SNP detection for each run. The CYP2D6 *5 gene deletion allele and duplicated alleles could not be assessed because of DNA fragmentation which results from paraffin fixation.

CYP2D6 metabolism definition

CYP2D6 phenotype groups were defined as previously published (11) where "extensive" metabolizers do not carry a null or reduced allele (EM/EM); those with 1 to 2 reduced alleles without a null allele (EM/IM, IM/IM); one null allele (PM/IM, PM/EM), and "poor" metabolizers, those with 2 null alleles (PM/PM). Information about the use of CYP2D6 inhibitors was unknown; however, the use of CYP2D6 inhibitors for the treatment of hot flashes was not recommended during the period of study enrollment on ABCSG8.

Study design and analysis plan

A matched case–control study was conducted to examine whether the odds of a disease event differed with respect to *CYP2D6* genotype. The definitions of a case and a control take into account the early release of the trial results in 2005 (17) as well as the opening of ABCSG16 [Secondary Adjuvant Long-Term Study With Arimidex; (SALSA); NCT00295620]. That is, time at risk for a disease event was truncated at the date of switch to anastrozole for those women on arm A who elected to switch from tamoxifen to anastrozole following the release the publication of the combined ABCSG8/ARNO95 analysis (17). For other patients, events after 5 years of therapy were not eligible for analysis given that ABCSG8 patients either enrolled onto ABCSG16 or information about extended adjuvant hormonal therapy after 5 years was not collected. This analysis plan was consistent with the approach in the published parent ABCSG8 trial (20). Using the definition of invasive disease free survival by Hudis and colleagues (21), a case was defined as a woman who had a documented local, regional, or distant recurrence of breast cancer; a contralateral breast cancer, or a second non-breast primary cancer or died from any cause during her time at risk. For a given case, 2 controls were selected using an optimal matching (22, 23) from among women randomized to the same treatment arm whose age at randomization was within 5 years for the case; whose primary treatment [modified radical mastectomy ± RT or breast-conserving surgery (BCS) + RT vs. BCS alone], tumor stage (I vs. II/III), and nodal status (positive vs. negative) was the same as the case and whose time at risk was longer than that of the case. In some situations, only one control was available for the case, either because matching criteria could not be met or adequate tissue was not available for genotyping of selected controls.

Conditional logistic regression modeling (CLRM) for matched triplets and pairs was used to examine whether the odds of a disease event differed with respect to CYP2D6 genotype in the following situations: (i) the first 5 years of treatment, for each arm separately, (ii) years 3 to 5 of treatment, for each arm separately, and (iii) in the first 2 years of treatment (when all patients were assigned tamoxifen). Two-sided $P < 0.05$ was considered statistically significant. Analysis was conducted using SAS (Version 9.2, SAS Institute Inc.). Assuming that 5% of the controls would be poor CYP2D6 metabolizers and the number of disease events would be 200, a 2-sided $\alpha = 0.05$ χ^2 test for the 2 to 1 matched odds ratio would have an 80% power for detecting an OR of 2.5 or greater.

Results

Characteristics of the patients

Of the 3,901 women enrolled in ABCSG8, 1,849 eligible patients were randomized to tamoxifen (arm A) and 1,865 eligible patients were randomized to the tamoxifen followed by anastrozole (arm B) There were 790 patients from arm A and 799 patients from arm B with tissue blocks available. There were 354 patients with a disease event and 1,235 potential controls among these 1,589 patients. However, 12 cases from arm A had an event after switching to anastrozole following early release of ABCSG8 results (17) and as such were ineligible. Of the remaining 342 cases, 23 (6.7%) cases were excluded from the analysis due to insufficient tissue in the block (9 cases) and unable to find a suitable control with adequate tissue (14 cases; Fig. 1). Thus, 319 cases were matched to 557 matched controls (2 controls/case for 238 cases and 1 control/case for 81 cases). The number of cases eligible for analysis based on therapy period (years 1 and 2, 3–5) are given in Table 1. Cases occurring after the first 5 years of therapy were not eligible for analysis as outlined above. The breakdown of the type of disease events is given in Table 2, and the characteristics of the cases and controls overall and by treatment arm are given in Table 3.

Genotype and allele frequency

The 5 *CYP2D6* SNPs associated with the CYP2D6 alleles: *4, *6, *10, and *41, and the *CYP2D6* *3 SNP were genotyped with a success rate of greater than 98% and 84%, respectively. The number of samples for each observed genotype and the corresponding minor allelic frequencies are shown in Table 4. The variant allele frequencies were similar to published reports in a predominantly Caucasian population, the predominant ethnic group in Austria (PhamGKB.org). Tests for HWE showed that *4 ($P = 0.07$), *6 ($P = 1.0$), and *10 ($P = 0.54$) alleles were within HWE, with some deviation for the *41 ($P = 0.009$) and rare *3 allele ($P = 0.003$).

Association of CYP2D6 phenotype with the likelihood of a disease event by treatment arm during the 5 years of therapy

In arm A during the 5 years of tamoxifen treatment, PM/PM [OR, 2.45; 95% confidence interval (CI), 1.05–5.73; $P = 0.04$] had a higher odds of a disease event relative to EM/EM (Table 5). There was also a trend for patients classified as IM/PM or EM/PM relative to EM/EM (OR, 1.67; 95% CI, 0.95–2.93; $P = 0.07$) but not in patients classified as EM/IM or IM/IM relative to EM/EM (OR, 1.23; 95% CI, 0.58–2.61; $P = 0.60$) to have a higher odds of a disease event. Conversely in arm B, no significant association was found between *CYP2D6* genotype and the likelihood of a disease event during the 5 years of treatment.

Association of CYP2D6 phenotype with the likelihood of a disease event in the first 2 years and last 3 years of treatment

Having observed marked differences between arms A and B in terms of the association *CYP2D6* genotype and the likelihood of a disease event over the 5 entire years of treatment, we conducted a secondary analysis to evaluate the nature of this association in the first 2 years of treatment when all patients received tamoxifen and in years 3 to 5 when patients on arm A continued tamoxifen and patients

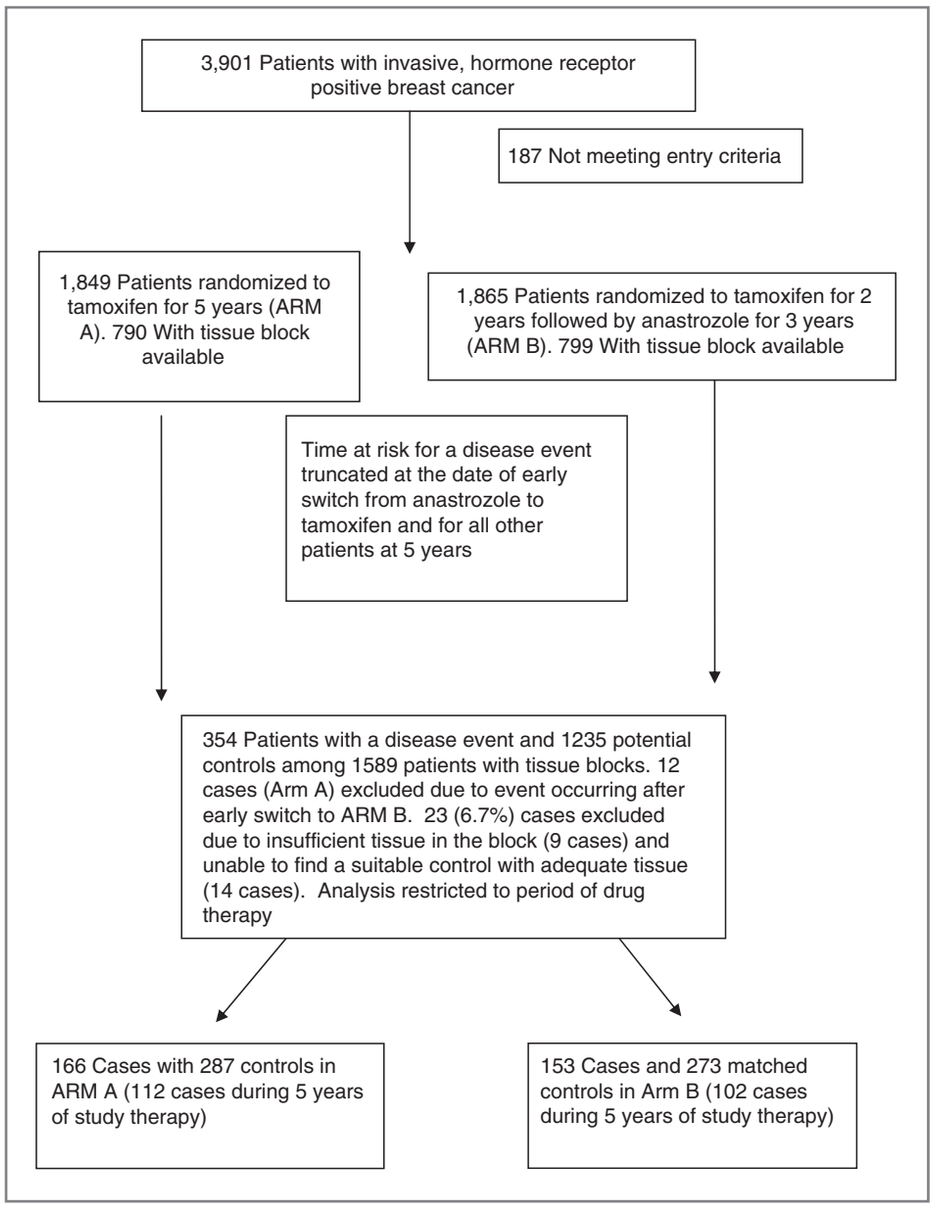

Figure 1. Consort diagram.

on arm B switched to anastrozole. There were a limited number of events during years 1 to 2 (Table 1); however, a similar nonsignificant higher odds of a disease event was observed for PM/PM relative to EM/EM in the first 2 years of tamoxifen for both arms: arm A: OR, 2.54; $P = 0.25$ and arm B: OR, 2.60; $P = 0.46$.

During years 3 to 5, for women on arm A who remained event-free during the first 2 years of tamoxifen therapy, PM/

Table 1. Number of cases analyzed in specific follow-up time periods relative to drug therapy

	Within 2 y	Years 3–5	Within 5 y	After year 5[a]	Total
Arm A	28	84	112	54	166
Arm B	41	61	102	51	153
Total	69	145	214	105	319

[a]Primary analysis excluded events occurring after 5 years (see Methods).

Table 2. First events among 319 cases

Event	*N* (%)
Local recurrence[a]	41 (12.9)
Distant recurrence[a,b]	90 (28.2)
Contralateral cancer	28 (8.8)
Second primary[b]	91 (28.5)
Death	78 (24.5)

[a]Seven patients experienced both local and distant recurrence as first event.
[b]Two patients experienced both distant recurrence and second primary as first event.

PM (OR, 2.40; 95% CI, 0.86–6.66; $P = 0.09$) and IM/PM or EM/PM (OR, 1.70; 95% CI, 0.91–3.17; $P = 0.09$) had a trend toward an increased odds of a disease event relative to EM/EM. In contrast, women on arm B who remained event-free during the first 2 years of tamoxifen, the odds of disease event for PM/PM (OR, 0.28; $P = 0.23$) and the IM/PM or EM/PM group (OR, 0.63; $P = 0.22$) were found to be nonsignificantly decreased during years 3 to 5 relative to EM/EM.

Discussion

Among the postmenopausal women with ER-positive breast cancer enrolled on ABCSG8 who were randomized to 5 years of tamoxifen (arm A), there was a significantly higher odds of a disease event for those with CYP2D6 PM/PM phenotype relative to those with the CYP2D6 EM/EM phenotype, but this was not observed in patients treated with anastrozole following tamoxifen (arm B). Moreover, in arm A, there was a strong trend toward a higher likelihood of a disease event (relative to EM/EM phenotype) in those that carried at least one poor allele (PM/IM and PM/EM) but not for patients without poor alleles (IM/IM and EM/IM). Because of the small number of patients with the IM/IM phenotype, no conclusions can be drawn about this group. The overall findings are consistent with pharmacokinetic data showing a stepwise reduction in endoxifen concentrations based on a number of PM alleles (8).

For ABCSG8 patients randomized to 2 years of tamoxifen followed by 3 years of anastrozole (arm B), *CYP2D6* genotype was not associated with the odds of a disease event. However, breaking the treatment period into the tamoxifen phase and the anastrozole phase, we found nonsignificant higher odds of a disease event among PM/PM relative to EM/EM in the first 2 years of tamoxifen similar to arm A but no evidence of significantly increased odds of a disease event during anastrozole treatment in years 3 to 5 for PM/PM phenotype relative to EM/EM phenotype. These data suggest that aromatase inhibitor use following tamoxifen negates or even reverses the higher likelihood of disease recurrence observed in patients with reduced CYP2D6 metabolism. The observation that reduced metabolism is detrimental only during the period of tamoxifen administration may explain

Table 3. Characteristics of the cases and controls

	Arm A		Arm B		Overall	
	Cases *N* = 166	Controls *N* = 287	Cases *N* = 153	Controls *N* = 270	Cases *N* = 319	Controls *N* = 557
Age, y, median (range)	66 (49–80)	66 (48–80)	69 (49–80)	67 (47–79)	68 (49–80)	66 (47–80)
Treatment, *n* (%)						
BCS without RT	29 (17.5)	45 (15.7)	25 (16.3)	45 (16.7)	54 (16.9)	90 (16.2)
BCS with RT	95 (57.2)	186 (64.8)	90 (58.8)	171 (63.3)	185 (58.0)	357 (64.1)
Mastectomy without RT	39 (23.5)	52 (18.1)	35 (22.9)	47 (17.4)	74 (23.2)	99 (17.8)
Mastectomy with RT	3 (1.8)	4 (1.4)	3 (2.0)	7 (2.6)	6 (1.9)	11 (2.0)
Tumor stage, *n* (%)						
I	102 (61.4)	181 (63.1)	94 (61.4)	165 (61.1)	196 (61.4)	346 (62.1)
II/III	64 (38.6)	106 (36.9)	59 (38.6)	105 (38.9)	123 (38.6)	211 (37.9)
Node status, *n* (%)						
Positive	66 (39.8)	108 (37.6)	56 (36.6)	93 (34.4)	122 (38.2)	201 (36.1)
Negative	100 (60.2)	179 (62.4)	97 (63.4)	177 (65.6)	197 (61.8)	356 (63.9)
Grade, *n* (%)						
I	29 (18.7)	64 (24.3)	29 (20.9)	65 (27.1)	58 (19.7)	129 (25.6)
II	126 (81.3)	199 (75.7)	110 (79.1)	175 (72.9)	236 (80.3)	374 (74.4)
Unknown	11	24	14	30	25	54
Her2 status, *n* (%)						
Positive	13 (8.1)	16 (5.8)	9 (6.1)	16 (6.1)	22 (7.2)	32 (5.9)
Negative	147 (91.9)	262 (94.2)	138 (93.9)	248 (93.9)	285 (92.8)	510 (94.1)
Unknown	6	9	6	6	12	15

Table 4. Number of observed genotypes and minor allele frequencies (*q*) for *CYP2D6* *3, *4, *6, *10, and *41 among the cases and controls

CYP2D6 allele	Number (of 876 samples)	Minor allele frequency
*CYP2D6 *3* (2549 delA)		$q = 0.01$
Wt/Wt	717	
Wt/*3	13	
*3/*3	2	
No call	144	
*CYP2D6 *4* (1846 G>A)		$q = 0.21$
Wt/Wt	558	
Wt/*4	271	
*4/*4	47	
No call	0	
*CYP2D6 *6* (1707 delT)		$q = 0.01$
Wt/Wt	831	
Wt/*6	23	
*6/*6	0	
No call	22	
*CYP2D6 *10* (100 C>T and 1846 G>A)[a]		$q = 0.03$
Wt/Wt	806	
Wt/*10	49	
*10/*10	1	
No call	20	
*CYP2D6 *41* (2988 A)		$q = 0.09$
Wt/Wt	735	
Wt/*41	125	
*41/*41	13	
No call	2	

[a]CYP2D6 *10 defined as 100T in absence of 1846A.

some of the controversy surrounding *CYP2D6* genotype and tamoxifen efficacy, given that switching from tamoxifen to an aromatase inhibitor is recommended (24). While early studies (before the routine use of aromatase inhibitors following tamoxifen) showed an association between CYP2D6 and tamoxifen efficacy (10, 11), multiple recent studies have been negative (25–27). However, a major concern of the negative studies is the lack of assurance that the confounding impact of a switch to an aromatase inhibitor was adequately considered.

Simon and colleagues have proposed a refined system for biomarker studies that incorporates a hierarchal level of evidence scale for tumor marker studies using archived specimens (28). This "prospective-retrospective" design stipulates that the clinical and pathologic characteristics of patients in the biomarker study be representative of patients in the parent trial and that a sufficient number of patients with archived tissue be included for adequate statistical power. Notably the ATAC *CYP2D6* analysis included 588 (18.9%) of the 3,116 women randomized to tamoxifen, and the clinical characteristics of genotyped patients differed significantly ($P < 0.005$) in multiple important clinical characteristics (e.g., CT, RT, hormone receptor status), both compared with nongenotyped UK patients and rest of the world patients. Furthermore, only 89 (17%) of the 535 distant recurrences were included in the tamoxifen *CYP2D6* analysis. Given the variants genotyped in ATAC, Schroth and colleagues estimated that more than 1,200 patients would be required to detect a hazard ratio of 1.85 between CYP2D6 poor (PM) and extensive metabolizer (EM) with 90% power (11). Therefore, the ATAC clinical analysis of *CYP2D6* was neither representative of the entire population nor adequately powered.

In contrast to ATAC, the ABCSG8 analysis of *CYP2D6* included 52% (214 of 408) of all first events occurring during the first 5 years (20). Our analysis plan was to match cases to 2 controls from a pool of 1235 ABCSG patients who did not have a disease event based on known prognostic factors and exposure time to increase the power to detect a smaller odds ratio. Because our study design focused on early events occurring during the period of drug administration (years 1–5), an unanswered question remains whether alterations in CYP2D6 metabolism affect the risk of late recurrences (after tamoxifen discontinuation).

Simon and colleagues additionally propose that for a "retrospective-prospective" design, the biomarker assay must be analytically and preanalytically validated for use with archived tissues (28). CYP2D6 enzyme activity (and therefore endoxifen concentrations) results from both *CYP2D6* germline variation, and the potency and duration of CYP2D6 inhibitors co-administered with tamoxifen. A

Table 5. Association between CYP2D6 phenotype and disease event during the 5 years of drug therapy in arms A and B

	Tamoxifen only (arm A)		Tamoxifen followed by anastrozole (arm B)	
	OR (95% CI)	*P*	OR (95% CI)	*P*
PM/PM relative to EM/EM	2.45 (1.05–5.73)	0.04	0.60 (0.15–2.37)	0.47
EM/PM and PM/IM relative to EM/EM	1.67 (0.95–2.93)	0.07	0.76 (0.43–1.31)	0.32
EM/IM and IM/IM relative to EM/EM	1.23 (0.58–2.61)	0.60	1.02 (0.52–2.01)	0.96

limitation of this and other prospective clinical trials evaluating tamoxifen is that germline DNA was never collected, and information about concomitant medications is unknown. Because paraffin-embedded tumors blocks which contain normal tissue are often collected, germline DNA may be extracted and used for genotyping. However, LOH involving chromosome 22q13.1, the location of the CYP2D6 gene (18, 19), has been noted in ER-positive tumors. Therefore, it is critical to assess for HWE, which states that both allele and genotype frequencies in a population remain constant assuming no new mutations, no selection, and random mating. Substantial departure from HWE may point to genotyping error or other biases. Notably, the BIG1-98 used tumor cores for *CYP2D6* genotyping and showed marked deviation from HWE for nearly all CYP2D6 alleles, including the most important *CYP2D6* variant (*4; HWE: $P = 10^{-91}$).

Within the ABCSG8 cohort, to obtain sufficient numbers of normal epithelial cells for the detection of germline genotypes, we extracted DNA from tissue sections that contained both normal and tumor tissue, and the *4, *6, and *10 alleles were within HWE. However, moderate deviation was observed for the rare *3 allele, as well as reduced metabolism alleles (Table 4). The latter observation may relate to somatic deletion of the CYP2D6 chromosomal locus (18, 19) or the presence of germline deletion of the entire CYP2D6 gene (*CYP2D6 *5*; ref. 29), leading to a deficit of observed heterozygotes. It should be noted, however, that the measured allele frequencies in this study were similar to previously published data from other European Caucasian populations (PharmGKB http://www.pharmgkb.org/index.jsp).

Prior studies evaluating the importance of adherence have indicated higher rates of nonadherence in patients without close follow-up (30) as well as in younger patients (<45 years; ref. 31). While adherence was not formally monitored, ABCSG8 enrolled postmenopausal women with a median age of and regular follow-up visits were required.

In summary, in the ABCSG8 clinical trial, the CYP2D6 PM/PM phenotype was associated with a higher likelihood of an early disease event in women treated with 5 years of tamoxifen but not in patients treated with sequential tamoxifen followed by anastrozole. Prospective studies are needed to determine whether altering the dose, the duration, or choice of adjuvant hormonal therapy based on *CYP2D6* genotype or the pharmacologic monitoring of endoxifen levels will improve the clinical outcomes of postmenopausal women with early-stage ER-positive breast cancer.

Disclosure of Potential Conflicts of Interest

M.P. Goetz reports that he has been a consultant for Gtx (not reimbursed). M. Gnant reports receiving research support from and serving as a consultant for AstraZeneca, Novartis, and Pfizer; and receiving lecture fees and honoraria for participation on advisory boards from AstraZeneca, Novartis, Sanofi-Aventis, Roche, Schering, Amgen, and Pfizer. No potential conflicts of interest were disclosed by the other authors.

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