# *CYP2D6* inherited variation and inhibiting medication use in relation to adverse breast cancer outcomes after tamoxifen therapy

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**Abstract**

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Tamoxifen (TAM) is widely used to reduce the risk of breast cancer (BC) recurrence and prolong disease-free survival among women with estrogen-sensitive breast cancers. TAM efficacy is thought to be attributable largely to the actions of its active metabolite, endoxifen, and must undergo biotransformation catalyzed by the cytochrome P450 enzyme, CYP2D6. Endogenous variation in CYP2D6 metabolic efficiency and use of medications that inhibit CYP2D6 activity are thought to increase the risk of adverse BC outcomes among women taking TAM. This cohort study of 960 women diagnosed with early-stage BC between 1993 and 1999 in the Seattle tri-county area examined the association between concomitant use of CYP2D6 inhibitors and adjuvant TAM and the risk of adverse BC outcomes, both overall and among women with specific CYP2D6 metabolic phenotypes. Six or more months of CYP2D6 inhibitor use concomitant with TAM was not associated with any appreciable change in risk of recurrence or second primary BC, regardless of a women's metabolic phenotype. These results are consistent with a number of other studies that have found no increased risk of adverse BC outcomes associated with CYP2D6 inhibition.

**Introduction** Tamoxifen (TAM), a selective estrogen receptor (ER) modulator, has been widely used to reduce the risk of breast cancer (BC) recurrence and prolong disease-free survival among women with nonmetastatic ER+ cancers. Clinical evidence indicates that five years of adjuvant TAM can lower the risk of recurrence and death due to ER+ BC by 41 and 33 percent, respectively.[1] Shorter durations of TAM use have been associated with lesser reductions in risk of recurrence and death,[2] while TAM use exceeding five years has been shown to afford further improvement in BC outcomes.[3] However, even among women who receive five years of adjuvant TAM therapy, approximately a third will experience a BC recurrence and more than a quarter will die from their cancer within 15 years.[4] Significant gaps persist in understanding the factors that influence TAM response and predicting which users are at the greatest risk for adverse BC outcomes.

TAM exhibits relatively weak binding for ERs and undergoes extensive biotransformation to its metabolites, *N*-desmethyl-tamoxifen, 4-OH-tamoxifen, and 4-OH-*N*-desmethyltamoxifen (endoxifen).[5] The major steps in TAM metabolism are catalyzed by a suite of cytochrome P450 (CYP) enzymes, including the product of the *CYP2D6* gene, which plays a major role in converting TAM's dominant primary metabolite to endoxifen (Figure 1). Endoxifen and 4-OH-tamoxifen are thought to be more pharmaceutically active than TAM due to their much higher binding affinity for the ER;[6 7] endoxifen, in particular, is found at 10-fold higher concentrations than 4-OH-tamoxifen in plasma and is thought to be TAM's primary active metabolite.[8]

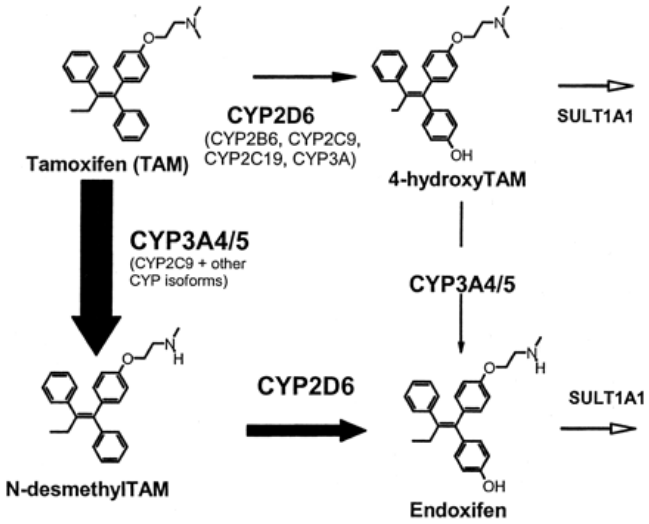


**Figure 1.** TAM metabolic pathway. Used without permission from Jin Y, et al. (2005).

One hypothesized basis for the inability of TAM to prevent recurrence of breast cancer in some women is concomitant use of medications that impede TAM's biotransformation into endoxifen. Co-administration of medications that interfere with CYP2D6 activity has been shown to lower plasma concentrations of endoxifen,[9 10] likely through competitive inhibition. These medications span several major drug classes, and some, including a number of selective serotonin reuptake inhibitors (SSRIs), are strongly inhibitive of CYP2D6 activity and are commonly used by breast cancer survivors for the treatment of depression, anxiety, pain, and of TAM's hormonal side effects.[11 12]

Endogenous metabolic efficiency has also been shown to impact the concentration of TAM and its active metabolites in blood, [13 14 15 16] and by extension, in breast and tumor tissue.[17] There are over 130 distinct allelic variants identified in the *CYP2D6* gene,[18] and up to 10 percent of the Caucasian population carries variant alleles implicated in near complete loss of enzymatic function.[19] Though other enzymes are involved in the biotransformation of TAM (Figure 1), an estimated 39% of the variability in plasma Z-endoxifen concentration is attributable to *CYP2D6* genotype, and in one study, 93% of those classified as poor metabolizers had endoxifen concentrations at or below the level recommended for broad ER inhibition.[20] Combined with the use of CYP2D6 inhibiting medications, women with poor- or intermediate-level metabolic efficiency may convert much less TAM into endoxifen, and be at greater risk for adverse BC outcomes.[21 22 23] However, results from epidemiologic studies of the risk of adverse BC clinical outcomes associated with CYP2D6 metabolism have been heterogeneous. Two secondary analyses of clinical trial data reported no increased risk associated with *CYP2D6* genotype,[24 25] and several studies have reported significantly

increased risks of adverse BC outcomes among women homozygous for a nonfunctional *CYP2D6* allele.[26 27] No single factor has been identified that can explain this heterogeneity,[28] but there are concerns about genotyping strategies, small sample sizes, length-biased sampling, and lack of control for prognostic factors and CYP2D6 inhibiting medication use.

This study assessed the association between concomitant use of CYP2D6 inhibiting medications and adjuvant TAM and risk of recurrences and second primary BCs among ER+ TAM users, using comprehensive approaches to *CYP2D6* genotyping and to quantifying durations of concomitant inhibitor and TAM use. Additional analyses assessed the potential interaction of concomitant CYP2D6 inhibitor use and metabolic phenotype, as well as the potential for confounding by indication: the only observed uses of moderate or strong CYP2D6 inhibitors in this cohort are of SSRIs indicated for the treatment of depression or anxiety, conditions that may be independently associated with adverse cancer outcomes.[29]

While previous studies have examined these associations in large and well-defined populations, the largest of these have limited their genotyping strategy to assessing only the *4 allele.[30] Our study employed a more comprehensive genotyping strategy for *CYP2D6*, allowing for a more refined categorization of metabolic phenotype. Furthermore, two recent clinical trials that reported on TAM efficacy in relation to *CYP2D6* phenotype used DNA extracted from formalin-fixed paraffin-embedded tumor tissues for genotyping.[31] These and other studies exhibited deviations from the expected Hardy-Weinberg equilibrium of genotype frequencies, suggesting the presence of genotyping errors or chromosomal instability. The *CYP2D6* locus is now known to be subject to loss of heterozygosity in breast tumor tissue, and thus results in tumor tissue do not provide an accurate reflection of the germline genotype relevant for hepatic drug metabolism;[32] our use of germline DNA therefore presents a significant advantage over previous studies.

Lastly, given that data on prescription medication use was abstracted from charts, we were able to quantify duration of concomitant medication use, which we hypothesize is the primary mechanism driving risk of adverse events, rather than relying on “ever use” of inhibitors to determine exposure. As prescribing practices for TAM have changed over the period in which women were diagnosed with BC and followed for adverse outcomes, this approach allows us to account for variation in TAM usage among the study population when assessing concomitant use.

## Methods

***Study design*** This retrospective cohort study examined the association between concomitant use of CYP2D6 inhibitors and TAM and the risk of second breast cancer events ([SBCEs] recurrences or second primary BCs) and of recurrences alone. Secondary analyses examined the potential for interaction with CYP2D6 metabolic phenotype and the possibility of confounding by pharmaceutically-treated depression or anxiety.

***Study setting*** The study was conducted within the Quilt Study cohort, which combined cases from three prior population-based case-control studies conducted in the Seattle tri-county area: the Women's Contraceptive and Reproductive Experiences Study (CARE); the Puget Sound Area Breast Cancer Evaluation Study (PACE); and the Electric Power and the Risk of Breast Cancer (EMF) Study. Between 76.5 and 80.6 percent of eligible cases (all ages and tumor types) enrolled into the three parent case-control studies. The Quilt Study collected data on pre- and post-diagnosis lifestyle factors, treatment, recurrences and other medical conditions in over 2,300 women aged 45-79 diagnosed with breast cancer in the Seattle three-county area between January 1993 and May 1999.

***Study population*** Women included in the present analysis were drawn from the Quilt Study cohort (269 from CARE, 464 from PACE, and 227 from EMF). The study population was restricted to women diagnosed with local or regional stage ER+ cancers (and no concurrent diagnoses with other cancers), whose charts were available for review, and who used adjuvant TAM (classified as use prior to any SBCE) for at least six months after primary diagnosis (N=960). Secondary analyses were restricted to the subset of women with genotyping data on *CYP2D6* (N=662, all from CARE or PACE studies) or to women diagnosed with or treated for depression or anxiety (N=382).

***Data collection*** Information on baseline patient characteristics, tumor characteristics, and on known risk factors for BC incidence was ascertained in the original case-control studies from the Cancer Surveillance System (CSS), the population-based SEER cancer registry serving the Seattle-Puget Sound area, and from structured in-person interviews. Following formation of the Quilt cohort, women were re-contacted for follow-up interviews regarding exposures and outcomes after their BC diagnosis, and their medical records were reviewed for information on cancer treatment, comorbidities, and recurrence history.

TAM and prescription medication use in targeted categories (including anti-depressant/anti-anxiety, anti-hypertensive, and NSAIDs) was abstracted from women's medical records. For each month following diagnosis and through the date of the last medical record reviewed, women were classified as either starting, stopping, or "still using" a medication in the absence of any indication of discontinuation.

From the available medication data, CYP2D6 inhibiting medications were identified and categorized with regard to inhibitor strength using the classifiers maintained by the U.S. Food and Drug Administration (FDA)[33] and Indiana University's Clinical Pharmacology Research Institute.[34] Both institutions classify inhibitor "strength" based on the change in *in vivo* plasma substrate concentration over time (measured as AUC) with and without co-administration of the inhibitor. Of the drugs observed in the data, these classifiers agreed on five out of six drugs classified as strong or moderate, and 100 percent on drugs classified as strong inhibitors. For the purposes of this study, medications were classified according to their highest indicated level of inhibition by either institution, and grouped into categories with increasing inhibitory strength, based on the expectation that effects were most likely to be observed in relation to use of strong or strong-or-moderate inhibitors.

**Table 1.** CYP2D6 inhibitors observed in study, by inhibitor strength and exposure classification

| | | Exposure classification | | |
|---|---|---|---|---|
| **Strength** | **Medications** | **Any inhibitor** | **Strong or moderate** | **Strong only** |
| Strong | *bupropion, fluoxetine, paroxetine* | X | X | X |
| Moderate | *duloxetine, sertraline* | X | X | |
| Weak or unknown | amiodarone, celecoxib, *citalopram, clomipramine*, diltiazem, *doxepin*, *escitalopram*, hydralazine, oral contraceptives, propafenone, verapamil | X | | |

*Italicized* medications are indicated for the treatment of depression and/or anxiety

Germline DNA from women in CARE and PACE was genotyped for nine SNPs in the *CYP2D6* gene in the Public Health Sciences' Molecular Epidemiology Laboratory at Fred Hutch. Combinations of these SNPs correspond to the different allelic variants described below (Table 2). Phenotypic diplotypes (EM/EM, EM/IM, EM/PM, IM/IM, IM/PM, and PM/PM) were collapsed into extensive, intermediate, and poor metabolizer categories based on the presence of at least one impaired allele (intermediate) or full impairment (PM/PM, poor).

**Table 2**. SNPs used in determination of allelic variants and metabolic phenotype.

| Variant | SNPs | Metabolic phenotype |
|---|---|---|
| *2 | rs1135840 and rs16947 | EM = Extensive metabolizer |
| *3 | rs35742686 | PM = Poor metabolizer |
| *4 | rs3892097 | PM |
| *5 (deletion) | deletion of *CYP2D6* | PM |
| *6 | rs5030655 | PM |
| *9 | rs5030656 | IM= Intermediate metabolizer |
| *10 | rs1065852 (without rs3892097) | IM |
| *35 | rs769258 | EM |
| *41 | rs28371725 | IM |

SBCEs were defined as the first local, regional, distant BC recurrence, or second primary BC occurring at least six months after the initial BC diagnosis; additional analyses were restricted to first recurrences. Data on recurrence was obtained from medical record review and interview, and data on second primary BC was collected from these sources as well as from CSS. In addition, for 45 women who died as a result of their breast cancer but for whom there was no record of distant recurrence, distant recurrence dates were imputed by subtracting the median duration between first distant recurrence and death date in cohort members who died with a preceding distant recurrence in their medical record (n=137) from the imputed woman's death date. If the imputed date was earlier than the last recorded disease-free date, then that later date was used instead.

***Analysis*** The relative risks of first recurrence and first SBCE associated with use of CYP2D6 inhibitors concomitant with TAM were estimated using Cox proportional hazards models. Women were followed from diagnosis for first SBCEs and first recurrence until their death, diagnosis with a non-breast cancer, date of last available medical record, or the end of follow-up on 29 March 2015.

Women who used TAM and a CYP2D6 inhibitor concurrently were considered exposed after six months of continuous or cumulative concomitant use and for the remainder of their follow-up time, given that a reduction in duration of "effective" TAM adjuvant therapy is expected to increase long-term risk of adverse BC outcomes. Subsequent analyses further restricted the exposed group to those who used respectively (i) strong or moderate CYP2D6 inhibitors and (ii) strong inhibitors alone, concomitant with TAM. Women who never used inhibitors concurrently with TAM (any strength) served as the reference group for all analyses.

Concomitant inhibitor exposure status and duration of adjuvant TAM were modeled as time-varying covariates, which allowed for updating of women's exposure status and duration of prior adjuvant TAM therapy at each event time. Analyses also adjusted for a number of pre-specified confounders: age at diagnosis (<55, 55-69, 70+), BMI prior to diagnosis (<25, 25-29.99, 30+ kg/$m^2$), tumor stage (local or regional), tumor grade (good, moderate, or poor differentiation, or undifferentiated), receipt of radiation, and receipt of chemotherapy. All categorical covariates were modeled as dummy variables, and duration of prior adjuvant TAM use was modeled as a time-varying, continuous variable.

A sub-analysis was conducted among women who underwent genotyping for the major *CYP2D6* alleles. Extensive metabolizers served as the reference group, with intermediate and poor metabolizers modeled using dummy variables. In the analysis of the interaction of concomitant inhibitor use and phenotype, phenotype main effect terms and terms for the interactions of concomitant inhibitor use category and phenotype were included in the model as dummy variables.

An additional sub-analysis was conducted among women who used medications for the treatment of depression or anxiety. Depression and anxiety may be independent risk factors for

adverse BC outcomes, and the primary analysis was unable to distinguish the potential impact of CYP2D6 inhibitors to treat these conditions from the possible effect of the conditions for which they are indicated. By conducting a sub-analysis among women treated for depression or anxiety and comparing outcomes with those who used CYP2D6 inhibiting medications, we hoped to distinguish the possible effects of these conditions on BC outcomes from any effect attributable to the interaction of these medications with TAM. This analysis was restricted to women with at least two records of use of "anti-depressant/anxiety, sleep" medications in their medical charts. This category of medications includes both CYP2D6 inhibiting and non-inhibiting medications and women entered the study in a time-varying manner following their first prescription (although two indications were ultimately required for inclusion in the sub-analysis). Only concurrent use of TAM and CYP2D6 inhibitors was considered in the assessment of exposure status; women who never used CYP2D6 inhibiting medications, or whose inhibitor use did not overlap with TAM use or overlapped for fewer than six months were considered unexposed. A Cox proportional hazards model was used to estimate the relative risk of SBCEs associated with concurrent CYP2D6 inhibiting medication use lasting at least six months.

**Results:** Nine-hundred sixty women from the Quilt cohort met study inclusion criteria. All women had non-metastatic ER+ cancers, had no other cancers at the time of breast cancer diagnosis, and had used TAM for at least six months.

Women who used any inhibitor concomitant with TAM for at least six months tended to be slightly older, were more likely to have been diagnosed between 1996-1997, had higher BMI, and were somewhat less likely to be postmenopausal than never users (Table 3). They were also more likely to be diagnosed at a regional stage, to receive chemotherapy, and had slightly longer durations of TAM use overall.

**Table 3**. Baseline demographic, tumor, and treatment characteristics, by total duration of concomitant medication use (never, fewer than six months, six or more months).

| | **Concomitant CYP2D6 inhibitor use among TAM users (N=960)** | | |
|---|---|---|---|
| ***Patient characteristics*** | **Never use (n=644)** | **Use <6 mos. (n=88)** | **Use 6+ mos. (n=228)** |
| Age at diagnosis | | | |
| *45-54 (%)* | 137 (21.3) | 17 (19.3) | 52 (22.8) |
| *55-69 (%)* | 289 (44.9) | 37 (42.1) | 86 (42.9) |
| *70-79 (%)* | 218 (33.9) | 34 (38.6) | 90 (39.5) |
| Year of diagnosis | | | |
| *1993-1995 (%)* | 227 (35.3) | 20 (22.7) | 63 (27.6) |
| *1996-1997 (%)* | 184 (28.6) | 33 (37.5) | 87 (38.2) |
| *1998-1999 (%)* | 233 (36.2) | 35 (39.8) | 78 (34.2) |
| BMI prior to diagnosis | | | |
| *<25 kg/m$^2$ (%)* | 329 (51.4) | 42 (47.7) | 110 (48.3) |
| *25-29.99 kg/m$^2$ (%)* | 199 (31.1) | 27 (30.7) | 64 (28.1) |
| *30+ kg/m$^2$ (%)* | 112 (17.5) | 19 (21.6) | 54 (23.7) |
| White (%) | 619 (96.1) | 84 (95.5) | 222 (97.4) |
| Menopausal status at diagnosis | | | |
| *Premenopausal (%)* | 66 (10.3) | 6 (6.8) | 28 (12.3) |
| *Perimenopausal (%)* | 32 (5.0) | 6 (6.8) | 17 (7.5) |
| *Postmenopausal (%)* | 546 (84.8) | 76 (86.4) | 183 (80.3) |
| ***Tumor and treatment characteristics*** | | | |
| Tumor stage at diagnosis | | | |
| *Local (%)* | 441 (68.5) | 54 (61.4) | 143 (62.7) |
| *Regional (%)* | 203 (31.5) | 34 (38.6) | 85 (37.3) |
| Grade at diagnosis | | | |
| *Well differentiated (%)* | 102 (17.7) | 14 (18.2) | 35 (16.6) |

| | | | |
|---|---|---|---|
| *Moderately differentiated (%)* | 261 (45.4) | 35 (45.5) | 94 (44.6) |
| *Poorly differentiated (%)* | 179 (31.1) | 23 (29.9) | 70 (33.2) |
| *Undifferentiated (%)* | 33 (5.7) | 5 (6.5) | 12 (5.7) |
| Primary radiation treatment (%) | 444 (69.2) | 61 (69.3) | 155 (68.0) |
| Primary chemotherapy (%) | 193 (30.1) | 28 (31.8) | 79 (34.8) |
| Total duration TAM use | | | |
| *6 months –<1 year* | 52 (8.1) | 7 (8.0) | 9 (4.0) |
| *1-<2 years* | 69 (10.7) | 7 (8.0) | 24 (10.5) |
| *2-<3 years* | 59 (9.2) | 8 (9.1) | 23 (10.1) |
| *3-<4 years* | 64 (9.9) | 13 (14.8) | 20 (8.8) |
| *4-<5 years* | 113 (17.6) | 20 (22.7) | 46 (20.2) |
| *5-<6 year* | 265 (41.2) | 32 (36.4) | 96 (42.1) |
| *6+ years* | 22 (3.4) | 1 (1.1) | 10 (4.4) |
| Total duration concomitant use | | | |
| *Never use* | 644 (100.00) | -- | -- |
| *1-<6 months* | -- | 88 (100.00) | -- |
| *6 months-<1 year* | -- | -- | 37 (16.2) |
| *1-<2 years* | -- | -- | 60 (26.3) |
| *2-<3 years* | -- | -- | 38 (16.7) |
| *3-<4 year* | -- | -- | 25 (11.0) |
| *4+ years* | -- | -- | 68 (29.8) |

There were 252 women who experienced a breast cancer recurrence or second primary cancer as their first event. Of these SBCEs, 19 were local recurrences, three were regional recurrences, 134 were distant recurrences (the date of which was imputed for 22 women), and 75 were second primaries; the remaining 21 SBCEs encompassed simultaneous diagnoses of multiple events (e.g., a local and distant recurrence diagnosed at the same time). Sixty-four women died and 158 were diagnosed with a non-breast cancer prior to any SBCE and were censored from the analysis at that time. In the analysis of time to first recurrence, women whose first event was a second primary BC were censored at the time of their second primary BC diagnosis.

Approximately 71.5 percent of the observed follow-up time occurred among never users of any concomitant CYP2D6 inhibitor, with an additional 8.1 percent among women who used a CYP2D6 inhibitor concomitant with TAM for fewer than six months, and the remaining 20.4 percent among women who used an inhibitor concomitant with TAM for six months or longer. Among women who used any CYP2D6 inhibitor concomitant with TAM for at least six months, there was a wide variation in durations of concomitant use, with more than half of the exposed women exceeding two years concomitant use. These patterns of medication use are paralleled in the analysis of the combined SBCE and recurrence endpoints.

Women who used inhibitors concomitant with TAM for fewer than six months were considered to be at low risk for medication-related treatment failure, and the HRs associated with short-term concomitant use were not significantly different than 1.0 across all inhibitor strength categories (data not shown). Overall, six or more months of CYP2D6 inhibitor use concomitant with TAM was not associated with any appreciable change in risk of recurrence or SBCE relative to never users (Table 4). Associations were not observed within any category of inhibitor strength. Concomitant inhibitor use appeared inversely related to the risk of the recurrence, but this association was not statistically significant.

**Table 4.** Association of 6+ months concomitant inhibitor use with recurrence and SBCE, by inhibitor strength

| | Recurrence | | | Recurrence or 2nd primary | | |
|---|---|---|---|---|---|---|
| **Inhibitor use category** | **Person-days** | **Events** | **aHR*** | **Person-days** | **Events** | **aHR*** |
| No inhibitor use | 2,430,155 | 126 | 1.00 (ref.) | 2,371,032 | 178 | 1.00 (ref.) |
| 6+ months any inhibitor | 693,732 | 38 | 0.83 (0.56,1.22) | 686,162 | 50 | 0.78 (0.55,1.10) |
| 6+ months strong or moderate | 382,222 | 20 | 0.74 (0.44,1.23) | 376,861 | 28 | 0.75 (0.48,1.18) |
| 6+ months strong only | 263,843 | 16 | 0.84 (0.48,1.46) | 259,594 | 23 | 0.90 (0.56,1.46) |

* Hazard ratios adjusted for age and BMI category, stage, grade, receipt of radiation and/or chemotherapy, and duration of adjuvant TAM

Six hundred and sixty-two women had genotyping samples available, of which 232 (35.0%) were classified as EM, 388 (58.6%) as IM, and 42 (6.3%) as PM. Table 5 indicates the breakdown of metabolizer phenotypes by ever use duration of any inhibitor concomitant with TAM.

**Table 5**. CYP2D6 metabolizer phenotype, by total duration of concomitant medication use.

| | **Concomitant CYP2D6 inhibitor use among TAM users with genotyping data (N=662)** | | |
|---|---|---|---|
| **Metabolic phenotype** | **Never use (n=423)** | **Use <6 mos. (n=66)** | **Use 6+ mos. (n=173)** |
| Extensive (%) | 146 (34.5) | 20 (30.3) | 66 (38.2) |
| Intermediate (%) | 248 (58.6) | 41 (62.1) | 99 (57.2) |
| Poor (%) | 29 (6.9) | 5 (7.6) | 8 (4.6) |

No association between metabolizer phenotype and length of disease-free survival was observed among never users in crude and adjusted analyses; results were consistent whether the three levels of metabolizer phenotype were compared or whether EMs were compared to the other two phenotypes combined (data not shown). Use of CYP2D6 inhibitors was not associated with an increased risk of SBCE regardless of a women's metabolic phenotype in a crude analysis (Table 6).

**Table 6**. Crude hazard ratios for the associations of 6+ months inhibitor use and CYP2D6 metabolic phenotype with SBCE

| **Inhibitor use category** | **Extensive metabolizers** | **Intermediate or poor metabolizers** |
|---|---|---|
| No inhibitor use | 1.00 (ref.) | 0.99 (0.66,1.47) |
| 6+ months any inhibitor | 0.82 (0.43,1.58) | 0.59 (0.32,1.07) |
| 6+ months strong or moderate | 0.87 (0.41,1.88) | 0.64 (0.31,1.34) |
| 6+ months strong only | 0.89 (0.38,2.12) | 0.83 (0.37,1.86) |

For our second sub-analysis, we identified 436 women with two or more indications of medication use for depression or anxiety in their medical charts, of which 189 were classified as having ever used inhibitors concurrently with TAM for six months or longer (190 never used these inhibitors concomitant with TAM). After accounting for delayed entry after first prescription use, 412 women were included in the analysis, and 114 SBCEs were observed during 1,181,511 person-days of follow up. Women who used any inhibitor concomitant with TAM for six months or longer and those who used strong or moderate inhibitors concomitantly for six

months or longer had a significantly reduced risk of recurrence or second primary (Table 7). Concomitant use of strong inhibitors was also associated with reduced risk of SBCEs, but this association was not statistically significant.

**Table 7.** Association of 6+ months concomitant inhibitor use with SBCE, by inhibitor strength, among women treated for depression or anxiety.

| | Recurrence or 2nd primary | | |
|---|---|---|---|
| **Inhibitor use category** | **Person-days** | **Events** | **aHR*** |
| No inhibitor use | 474,225 | 53 | 1.00 (ref.) |
| 6+ months any inhibitor | 531,946 | 42 | 0.57 (0.36,0.90) |
| 6+ months strong or moderate | 377,182 | 28 | 0.55 (0.32,0.92) |
| 6+ months strong only | 259,774 | 23 | 0.65 (0.38,1.14) |

* Hazard ratios adjusted for age and BMI category, stage, grade, receipt of radiation and/or chemotherapy, and duration of adjuvant TAM

**Discussion** Overall, this study found no increased risk of an adverse BC outcome associated with concomitant use of CYP2D6 inhibiting medications and TAM. No association with the risk of any SBCE or recurrence specifically was observed for any level of inhibitor strength. Furthermore, no association with metabolic phenotype was observed nor was there evidence for an interaction of metabolic phenotype and concomitant inhibitor use.

The main results of this study are consistent with a number of other observational studies that have observed no association between SSRIs and other CYP2D6 inhibitors, CYP2D6 metabolic phenotype, or CYP2D6 inhibition broadly on adverse BC outcomes.[35 36 37 38 39] Despite evidence that both use of CYP2D6 inhibiting medications and metabolizer phenotype are associated with reduced plasma endoxifen levels, no observational study has observed a significant association between inhibitor usage and clinical BC outcomes.

The present study is limited by a small number of events among exposed women, leading to imprecise hazard ratio estimates. We hypothesized that concomitant use lasting fewer than six months was unlikely to affect long-term risk of adverse events and so chose six months as our threshold for exposure, but the small number of events among women with longer exposure also prevented us from assessing any association due to longer periods of CYP2D6 inhibition. The timing of inhibition following BC diagnosis and duration of subsequent unopposed TAM use may also affect long-term risk of adverse outcomes, but these factors could not be examined in this study.

If anything, there was suggestion of a reduced risk of adverse BC outcomes in relation to concomitant inhibitor use, although the confidence limits for the risk estimates did not exclude the null. This is suggestive of some degree of “healthy user” or possible “healthy adherer” bias among long-term users of CYP2D6 inhibiting medications, which in this study are indicated primarily for the treatment of depressive and cardiac conditions. This effect involves the propensity for those that seek treatment for chronic conditions to make other healthy lifestyle choices, such as adherence to other medications, maintaining a healthy weight, and others.[40] The observed protective effect of inhibitor use could be partially attributable to the influence of these confounders, as well as latent variables like social support and care access that were not controlled for in this analysis. Although not specified as confounders *a priori* in this analysis, the distribution of education and income appeared similar when comparing never concomitant inhibitors users to women who were ever exposed for six months or longer. Never concomitant users had lower incomes on average in the year prior to diagnosis than exposed women, but were slightly more likely to have graduated college.

Recent commentaries have suggested that though TAM and its metabolites are expected to greatly overwhelm estrogens in competition for tumore ER binding sites, deleterious effects of concomitant inhibitor use or CYP2D6 phenotype on endoxifen concentrations may be most pronounced among premenopausal women, who have higher levels of endogenous estrogens than postmenopausal women.[41] We did not observe any difference in effect between postmenopausal and pre- or peri-menopausal women in an exploratory analysis (data not shown).

This analysis relied on medical record data on medication use and thus is limited to prescription CYP2D6 inhibitors and is likely less reliable than insurance claims or pharmacy fill data for accurately assessing medication use. Our system of medical record abstraction relied on the coding of drug use as starting, stopping, or continuing ("still using"), a status that was assumed in the absence of any record of stopping after a medication had been initiated. This approach may not have adequately captured sporadic use of a medication and may overestimate exposure duration. In addition, while all inhibitors classified as having strong or moderate activity by the FDA and Indiana University classification systems are prescription medications likely to appear in medical records, several weak inhibitors are not prescription medications (e.g., Echinacea), and therefore may result in some misclassification of exposure and attenuation of effect estimates towards the null.

Our study used a three-armed approach to ascertaining SBCEs. Recurrences and second primary BCs were identified through comprehensive medical record reviews and phone interviews with women or their proxies, if deceased. The CSS provided additional information on second primary BCs (recurrences are not currently collected in the SEER system). Despite our comprehensive approach, we cannot rule out the possibility of having not captured all SBCEs, in part because some women may not seek diagnosis or treatment for a recurrence and medical records may not cover their entire follow up period. Almost a quarter (24.7%) of women in this analysis who are reported to have died from their breast cancer did not have any record of having had a distant metastasis. Rather than treat them as non-recurrent before death, we used the median duration between recurrence and death to impute distant recurrence dates. We acknowledge this is a fairly simplistic approach to handling missing data, and does not take into account other factors that may allow a more refined imputation of recurrence dates for these women. Of greater concern is that imputation of distant recurrence dates using the median duration between distant recurrence and BC death in the rest of the cohort may ascribe the incorrect temporal sequence for exposure and outcome to a number of women whose actual recurrence occurred before the imputed recurrence. Use of this later imputed date for assessing prior exposure status may result in use of concomitant medications that appeared to precede recurrence where medication use and particularly, SSRI use, actually occurred in response to a recurrence. It is also possible that some deaths among women with BC are not actually caused by their BC, but may be “over coded” as BC-specific deaths, resulting in imputation of recurrences that never occurred. Excluding distant recurrences that were first SBCEs from the analysis did not alter the results (data not shown).

The requirement that women survive to participate in the original case-control study interview and complete at least six months of adjuvant TAM therapy may have resulted in overestimation of survival following a BC diagnosis and diminishes the generalizability of the results. One concern is that women with survival times less than six months from diagnosis could not have been classified as inhibitor users for six or more months even had we not required six months of TAM use, a requirement that was chosen to exclude women who had very aggressive cancers and possibly undiagnosed metastases, although we also restricted the

study on the basis of stage and ER status to eliminate a large portion of the most aggressive tumors. Also, given that we allowed exposure classification (including duration of concomitant use) and prior duration of TAM use to change over time and that the hazard ratio takes a conditional interpretation, comparing hazards at each event time *among* women who have survived event-free for the same amount of time, we are not concerned about immortal time bias related to survival, assuming women are not misclassified with regard to exposure status.

Lastly, despite concerns that depression or anxiety may have been independently associated with risk of adverse BC outcomes, no increased risk associated with concomitant use of CYP2D6 inhibitors and TAM was observed among women with medically treated depression or anxiety. In fact, concomitant use of any inhibitor or strong and moderate inhibitors was associated with a significantly reduced risk of SBCEs in this population. Though risk estimates from the analysis of the complete study population were suggestive of a reduced risk of SBCEs, they were not statistically significant, and we are unsure why a reduced risk would be detected among women with treated depression, particularly since it controls to some degree for healthy user/adherer bias (all women were required to have two indications in their medical record of use of medications for depression or anxiety for inclusion). CYP2D6 medication use alone (regardless of TAM use) was not associated with any change in the risk of adverse outcomes in an exploratory analysis. It is possible that this finding is simply due to chance, but could be examined in future studies.

Overall, this study found no evidence for increased risk of adverse BC outcomes among women who used CYP2D6 inhibiting medications concomitant with TAM or women with impaired CYP2D6 metabolic efficiency, with or without concomitant use of inhibitors. These findings are consistent with a number of recent observational studies, and suggest that there is little evidence at this time to support avoidance of CYP2D6 inhibiting medications while on TAM adjuvant therapy. Findings were similar across all levels of inhibitors strengths and among pre- or peri- and postmenopausal women. It is possible that accounting only for *CYP2D6* inherited variation and CYP2D6 inhibiting medication use does not fully capture impairment of TAM biotransformation, since additional enzymes are involved in TAM metabolism, though medication use and metabolic phenotype have been shown to correlate strongly with plasma endoxifen concentration.[42 43 44 45] A more comprehensive approach that can account for the entire TAM metabolic pathway may be needed to fully capture the effect of medication use and endogenous metabolic phenotypes on risk of adverse BC outcomes.

Others have proposed that even with reduced metabolic efficiency or pharmaceutical inhibition of CYP2D6, TAM and its metabolites would still greatly overwhelm estrogens in competition for ER binding sites in estrogen-sensitive tumor cells at standard doses, and so effects on BC survival would be minimal.[46] Early evidence indicating greater risk among women with CYP2D6 inhibition may be a result of “regression to the mean;” several meta-analyses have indicated that effects on disease-free survival are likely to be small, although results of meta-analyses also vary depending on inclusion criteria.[47 48 49 50 51 52] As such, large prospective studies may be needed to firmly establish the effects of CYP2D6 inhibition on BC outcomes, particularly among women most likely to use TAM (TAM is not typically recommended for postmenopausal women since the approval of aromatase inhibitors). Further studies may also be needed to validate the reduced risk of adverse outcomes observed among women with pharmaceutically-treated depression or anxiety who used CYP2D6 inhibitors concomitant with TAM.

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