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# Clinical epidemiology and pharmacology of CYP2D6 inhibition related to breast cancer outcomes

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## Abstract

Adjuvant tamoxifen therapy of breast cancer patients with estrogen receptor-positive tumors reduces the rate of breast cancer recurrence by approximately a half. Tamoxifen is metabolized by several polymorphic enzymes, including cytochrome P450 2D6 (CYP2D6), to more active metabolites. We have reviewed the clinical pharmacology of tamoxifen and evaluated the evidence from clinical epidemiology studies regarding the association between CYP2D6 inhibition and tamoxifen effectiveness. We conclude that the impact of CYP2D6 inhibition on tamoxifen effectiveness is likely to be null or small, at least in the populations studied so far. Understanding the effect of variations in tamoxifen metabolism on breast cancer outcomes, if any, will likely require a broader perspective, including examination of the complete metabolic pathway and subgroups of patients with other markers of potentially poor tamoxifen response.

### Keywords

breast cancer; breast cancer recurrence; cytochrome P450 2D6; selective serotonin reuptake inhibitors; tamoxifen

Tamoxifen, a selective estrogen receptor (ER) modulator, was a trailblazer for personalized cancer therapy. In stage I, II and III breast cancer patients with tumors that express the estrogen receptor (ER+) – approximately two out of three patients – tamoxifen reduces, by half, the rate of breast cancer recurrence and the risk of mortality by a quarter [1] . Current editions of the major treatment guidelines all reach the same recommendations [2,3,201] . ER+ premenopausal patients should receive tamoxifen for 5 years; aromatase inhibitors (AIs) are contraindicated outside of clinical trials. ER+ postmenopausal patients should receive AI either as initial therapy or in sequence with tamoxifen. Postmenopausal women with contraindications to AI, or who decline AI, should receive 5 years of tamoxifen therapy. Thus, tamoxifen remains fundamental in adjuvant breast cancer therapy.

Despite its impressive therapeutic effects, and more than 30 years history as a cancer therapy, recurrent disease refractive to endocrine therapy develops in some tamoxifen-treated women and they succumb to their cancer. Women with seemingly identical clinical and prognostic factors at breast cancer diagnosis, and who are treated with the same tamoxifen regimen, can have very different outcomes.



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The era of personalized medicine is rapidly evolving [4], and developments related to tamoxifen treatment of breast cancer patients are no exception. A longstanding research field has focused on identifying predictive markers of tamoxifen resistance [5–7] . Most recently, modification of tamoxifen's effectiveness by functional variants in the enzymes that activate and deactivate the parent drug, or by inhibition of these enzymes by other prescription drugs, has received a lot of attention in the evolution of tamoxifen personalization [8,9].

Cytochrome P450 (CYP) enzymes (CYP2D6, CYP2C19, CYP2C9, CYP3A4 and CYP3A5) metabolize tamoxifen to more active forms [10] , each with its own binding affinity to the ER. The compounds that most efficiently bind to the ER are hydroxylated at tamoxifen's four-carbon [11]. The strength of ER affinity predicts the anti-tumor response [12] . The major enzymes involved in solubilizing tamoxifen metabolites into excretable forms are uridine 5′-diphospho-glucuronosyltransferases (UGTs; primarily UGT1A8, UGT1A10, UGT2B7 and UGT2B15) [13] and sulfotransferases (primarily SULT1A1) [14]. All of the enzymes in this pathway are polymorphic, and the changes in function related to genotypes contribute to interindividual differences in tamoxifen metabolite concentrations in the serum [14–16].

Despite the complexity of this pathway, most clinical epidemiology studies examining the associations between gene variants and breast cancer outcomes have focused on only one player in this intricate pathway, namely CYP2D6. The current article therefore has two objectives. First, we will evaluate the evidence to date regarding the association between CYP2D6 inhibition and tamoxifen effectiveness, by reviewing the clinical pharmacology of tamoxifen and meta-analyzing the clinical epidemiologic studies. Second, we suggest that understanding the effect of variations in tamoxifen metabolism on breast cancer outcomes, if any, will require a broader perspective than that taken so far.

## Methods

### Search strategy & selection criteria

For our review of the association between CYP2D6 inhibition and tamoxifen effectiveness, we searched for the terms 'tamoxifen' and 'CYP2D6' in PubMed. No language restrictions were imposed. All papers published or presented as abstracts through 21 March 2011 regarding the association between *CYP2D6* gene variants or drug–drug interactions and the risk of breast cancer recurrence or mortality were reviewed to determine whether their results should be included. Citations included within the selected scientific papers or other reference sources were also used to locate other articles, for example, conference abstracts.

### Meta-analyses

We created four separate meta-analytical models. The first two focused on population-based studies associating concurrent use of weak or strong CYP2D6 inhibitors (selective serotonin reuptake inhibitors [SSRIs]) and breast cancer recurrence or breast cancer-specific mortality in tamoxifen-treated women. The third and fourth models focused on population-based studies associating *CYP2D6* inherited mutations and breast cancer recurrence or breast cancer-specific mortality in tamoxifen-treated women. The first of these compared recurrence risks of homozygote and heterozygote carriers of decreased-function alleles with homozygote carriers of the corresponding full-function allele, and the second compared recurrence risks of homozygote carriers of decreased-function alleles with homozygote or heterozygote (only Xu *et al.* [17]) carriers of the corresponding full-function allele. For these analyses, we searched all scientific papers or conference abstracts to obtain study-specific effect estimates for the association of inheritance of at least one variant allele of *CYP2D6* (either **4* or **10* ) with breast cancer recurrence or mortality. When studies presented

associations for heterozygote and homozygote variant alleles separately, we estimated an inverse variance weighted average of these two associations, which was then used in the first of the genetic meta-analytic models.

### Statistical analysis

We used random-effects meta-analytic models to generate summary effect estimates. In all cases, estimates from fixed-effects models were similar. We constructed funnel plots to evaluate publication and other sources of bias in the meta-analyses. These plots, which are available from the authors, showed no evidence of publication bias. All analyses were performed using STATA software, version 11.0 (StataCorp LP, College Station, TX, USA). All statistical tests were two-sided.

## Results

### Pharmacological evidence: CYP2D6 inhibition & the profile of tamoxifen metabolite concentrations

Tamoxifen is metabolized mostly in the liver, where it primarily undergoes 4-hydroxylation [18,19] and *N*-demethylation reactions (FIGURE 1) [20]. CYP enzymes metabolize tamoxifen and *N*-desmethyl-tamoxifen to 4-hydroxytamoxifen and 4-hydroxy-*N*-desmethyl tamoxifen (sometimes called endoxifen), respectively [21] . As noted previously, these metabolites bind the ER approximately 100-fold more readily than their respective parent molecules, so they are important modulators of the ER pathway [11]. Since 4-hydroxy-*N*-desmethyl-tamoxifen is present at a threefold to fivefold higher concentration in the serum than 4-hydroxytamoxifen, it is a key tamoxifen metabolite.

One phenotypic categorization of a person's capacity to metabolize tamoxifen depends on the ratio of the steady-state concentration of the administered drug, or its *N*-demethylated metabolite, to the steady-state concentration of its 4-hydroxylated metabolites [14,22] . Depending on this ratio, patients can be classified according to their ability to metabolize tamoxifen as 'poor metabolizers', 'intermediate metabolizers', 'extensive metabolizers' or 'ultra-rapid metabolizers'.

As depicted in FIGURE 1, CYP2D6 catalyzes activation of *N*-desmethyltamoxifen to 4-hydroxy-*N*-desmethyltamoxifen [23] and accounts for approximately 45% of the activation of tamoxifen to 4-hydroxytamoxifen [24] . More than 90 polymorphic variants of CYP2D6 have been identified, some of which reduce or eliminate CYP2D6 activity [202] and therefore affect the ability to metabolize tamoxifen [14,15,23,24]. As a second approach to phenotypic categorization, 'poor metabolizers' can be categorized as those with two nonfunctional CYP2D6 alleles, 'intermediate metabolizers' as those with one functional allele, 'extensive metabolizers' as those with two normal alleles, and 'ultra-rapid metabolizers' as those with multiple allelic copies of functional alleles and thus excess metabolic capacity. *In vivo* evidence supports correlated categorization of phenotype by these two approaches [22] . Further complicating the phenotypic categorization is the potential for drug– drug interactions to affect the concentration profile of tamoxifen metabolites [15,23,25]. While the affect of this drug–drug interaction would be apparent using the first strategy for phenotypic characterization, it would not be incorporated into the second strategy, which relies only on genotype to infer phenotype.

Regardless of the strategy used to categorize phenotype, it is clear that interindividual differences in the serum concentration of tamoxifen metabolites – either due to inhibition of the enzyme active site by CYP2D6-inhibiting drugs or to the inheritance of variant alleles in the genes coding for the metabolizing enzymes – could modulate the effectiveness of tamoxifen treatment. While the biologic rationale for this idea seems compelling, it is

counterbalanced by further consideration of the mechanism of tamoxifen's action. As mentioned at the outset, tamoxifen is a selective ER modulator – it acts in concert with its metabolites by competing with estrogen for binding to the tumor's ERs. As noted by Jordan almost 30 years ago:

> *"…the metabolic activation of tamoxifen is an advantage rather than a requirement for anti-estrogenic activity. The action of tamoxifen* in vivo *is the net result of the individual actions of the parent compound and its metabolites competing for the occupation of receptors within target tissues and tumors"* [26].

When administered at the standard dose of 20 mg/day, tamoxifen and its metabolites overwhelm estrogen in this competition, thereby occupying almost all of the available receptor binding sites and depriving the tumor of growth stimulation mediated by the estrogen–ER complex. The ER-binding activity of the drug and its metabolites on average outweighs the activity of estradiol by more than 5000 to one in post-menopausal women and by more than 500 to one in premenopausal women [8]. A fewfold reduction in the concentration of one of the active metabolites would be expected to have little effect on the competition.

## Tamoxifen effectiveness in the presence of a potential drug–drug interaction: the example of SSRIs inhibiting CYP2D6 function

Although tamoxifen is generally well tolerated, its anti-estrogenic and estrogenic actions sometimes produce mild-to-severe side effects, including hot flashes and vasomotor symptoms, induction or exacerbation of depression (sometimes also a pre-existing condition or a consequence of breast cancer diagnosis), venous thromboembolism and endometrial cancer [27–34]. SSRIs can provide effective clinical control of the depressive and vasomotor side effects and are therefore sometimes prescribed to women undergoing tamoxifen treatment. Both SSRIs and tamoxifen are primarily metabolized by CYP2D6 [21,35,36]. The net result can be competitive inhibition or direct inhibition of tamoxifen metabolism, resulting in a reduced plasma concentration of 4-hydroxy-*N*-desmethyltamoxifen [15,23] . Therefore, the safety of concurrent use of the two medications has come under scrutiny [37,38].

Selective serotonin reuptake inhibitors inhibit CYP2D6 to varying degrees [36]. Paroxetine and fluoxetine are the strongest inhibitors, with paroxetine irreversibly inhibiting CYP2D6 activity, whereas fluvoxamine and citalopram are weaker inhibitors. Reduced plasma concentrations of 4-hydroxy-*N*-desmethyltamoxifen have been reported in women who used paroxetine or fluoxetine concomitantly with tamoxifen, intermediate concentrations among women treated with the weaker CYP2D6 inhibitors sertraline and citalopram, and little effect among those using the selective serotonin norepinephrine reuptake inhibitor (SSNRI) venlafaxine – a weak CYP2D6 inhibitor [15,23,39,40].

A total of 11 clinical epidemiology studies have investigated the association between taking SSRI medications and breast cancer outcomes among tamoxifen-treated breast cancer patients [41–51] , with fairly heterogeneous results. Three of these studies have overlapping patients and follow-up time [42,46,47], therefore, we included only the most relevant report in the meta-analyses [47]. FIGURE 2A & B show the results of our meta-analyses of the included studies, organized by presupposed strength of CYP2D6 inhibition (Figure 2A shows weak inhibitors, such as citalopram; FIGURE 2B shows strong inhibitors, such as paroxetine). The study with greatest weight was Kelly *et al.*, as indicated by the relative area of its squares on the graphs [45] . The summary random-effects estimate associating breast cancer recurrence with concomitant use of tamoxifen and a weak CYP2D6 inhibitor was 1.05 (95% CI: 0.91–1.22). The summary random-effects estimate associating breast cancer recurrence with concomitant use of tamoxifen and a strong CYP2D6 inhibitor was 1.14

(95% CI: 0.88–1.46). These summary estimates indicate that the overall effect of concurrent use of SSRIs and tamoxifen on breast cancer recurrence is null or small.

## Tamoxifen effectiveness in the presence of a potential gene–drug interaction: the example of genetic variants inhibiting CYP2D6 function

As noted previously, and depicted in FIGURE 1, the metabolic pathway of tamoxifen involves a large set of enzymes. The enzymes that participate in 4-hydroxylation are most likely to influence the concentration profile of tamoxifen's metabolites. Because CYP2D6 is almost entirely responsible for 4-hydroxylation of the most abundant metabolite (*N*-desmethyltamoxifen), variants that reduce or eliminate CYP2D6 function may have the greatest effect on the profile of metabolite concentrations. Reduced plasma concentrations of 4-hydroxylated metabolites have been reported in women who carry two reduced-function alleles, and intermediate concentrations have been reported among women who carry one reduced-function allele [14,15,17].

To date, 24 studies have investigated the association between CYP2D6 inhibition by genetic variants and the effectiveness of adjuvant tamoxifen treatment [17,44,52–73] . Two of these studies [53,72] are updates of earlier reports [44,54,59], with overlapping patients and follow-up time, so we only included the updated reports in the meta-analyses. We were unable to extract estimates of association from two studies [61,73] , both of which characterized their results as null. As previously reviewed by our group [8] , the results of these studies are widely heterogeneous ($p < 0.001$, for test of homogeneity) and almost evenly distributed either side of the null. The precision of many of the studies was very poor, as portrayed by the width of the confidence intervals in FIGURE 3A & B. Taken together, the heterogeneity of results and symmetric dispersion about the null should caution against any strong conclusion regarding a causal association.

Our quantitative meta-analysis of these studies first compared the risk of breast cancer recurrence or mortality with the inheritance of at least one nonfunctional *CYP2D6*4* or *CYP2D6*10* allele. The results are presented in FIGURE 3A. The effect estimates reported in the studies range from 0.52 to 6.48, with eight reporting associations below the null and ten reporting associations above the null, and 11 out of the 18 studies including the null in their 95% confidence intervals. The summary random-effects estimate associating breast cancer recurrence with variant CYP2D6 genotype was 1.23 (95% CI: 0.97–1.55). The studies with the greatest weight, as indicated by the relative areas of the squares in the graph, are those with associations nearest to the null. An important exception is the updated analysis [72] of the Goetz [44] and Schroth [59] cohorts. It appears, however, that the association may have been null in the newly accumulated person-time included in the second analysis [74].

Our next quantitative meta-analysis compared the risk of breast cancer recurrence or mortality with the inheritance of two nonfunctional *CYP2D6*4* or *CYP2D6*10* alleles. The results are presented in FIGURE 3B. The effect estimates reported in the studies range from 0.57 to 9.52, with two reporting associations below the null and seven reporting associations above the null, and five out of the nine studies including the null in their 95% confidence intervals. The summary random-effects estimate associating breast cancer recurrence with variant *CYP2D6* genotype was 1.64 (95% CI: 1.09–2.47). This result suggests a non-null association between CYP2D6 inhibition among carriers of two reduced-function alleles and recurrence risk. We note, however, that the three studies with the strongest associations have important design flaws [8]. The two studies with the strongest associations were studies of *CYP2D6*10* [17,53], which is a reduced-function allele, but does not eliminate CYP2D6 function. If CYP2D6 inhibition increases the risk of recurrence among tamoxifen-treated patients with two reduced-function alleles, then one would expect the strongest associations

to be observed among carriers of two *CYP2D6*4* alleles, because that variant eliminates CYP2D6 function.

## Discussion

### Review of the evidence regarding CYP2D6 inhibition

In both the overview of trial results comparing approximately 5 years of tamoxifen against placebo [1], and in the Arimidex, Tamoxifen, Alone or in Combination trial results comparing approximately 5 years of aromatase inhibitor against tamoxifen [75,76], the 5-year risk of breast cancer recurrence among tamoxifen-treated patients ($R_T$) was approximately 15%. This comparability suggests that the trial populations are approximately exchangeable [77]. If we assume that the recurrence risk in any subgroup of tamoxifen-treated patients ($R_i$) cannot be greater than the recurrence risk in placebo-treated patients ($R_P$) or less than the recurrence risk in aromatase-treated patients ($R_{AI}$), then the comparison of any two subgroups ($R_1/R_2$) cannot be less than the ratio ($R_{AI}/R_P$; the effect of AI vs placebo) or greater than the ratio ($R_P/R_{AI}$; the effect of placebo vs AI). From their respective analyses at 5 years of follow-up, $R_{AI}/R_T = 0.79$ [75] and $R_T/R_P = 0.59$ [1]. We can therefore write the equation as seen in Box 1.

Any ratio of risks, rates or hazards in tamoxifen-treated subgroups outside these limits suggests an implausible point estimate or requires that the biomarker used to categorize the subgroups has both predictive and prognostic value. To date, no one has postulated a direct effect of CYP2D6 inhibition on the risk of breast cancer recurrence. The only effect, if any, is thought to be mediated through modulation of the profile of metabolite concentrations. CYP2D6 inhibition is therefore hypothesized to predict response to tamoxifen therapy, but not to have any prognostic value in itself. Valid estimates of the relative risk of recurrence in the tamoxifen-treated subgroups created by categorization of CYP2D6 inhibition should be expected, therefore, to fall into the range 0.47–2.15. Of the studies included in our meta-analyses, six out of seven studies (depicted in FIGURE 2A), eight out of nine studies (depicted in FIGURE 2B), 12 out of 17 studies (depicted in FIGURE 3A) and six out of nine studies (depicted in FIGURE 3B) yielded point estimates that fell into the expected range. Confidence intervals of all studies overlapped the range. While some of the incongruity between the results and the expected strength of estimated associations may be explained by chance, the heterogeneity of results and deviation from expectation merits further consideration.

Box 1. Estimated limits on the ratio of recurrence risks between any two tamoxifen-treated subgroups, assuming only a predictive effect of the marker used to create the subgroups

$$0.47=(R_{AI}/R_T)*(R_T/R_P)=R_{AI}/R_P \le R_1/R_2 \le (1/[R_T/R_P])*(1/[R_{AI}/R_T])=R_P/R_{AI}=2.15$$

An initial consideration is the potential for the potency of CYP2D6 inhibition to vary from study to study. For example, differences in physicians' preferences for prescribing specific antidepressants may vary geographically. Citalopram and escitalopram were the most frequent SSRI prescriptions in the Danish study [47], paroxetine and fluoxetine predominated in a North American study [41], and moderate/strong and weak CYP2D6 inhibitors were prescribed in approximately equal proportion in a UK study [48]. Citalopram inhibits CYP2D6 less than most other SSRIs, but despite the variability in prescribing

patterns, all three studies yielded near-null results. Similarly, *CYP2D6*4* knocks out CYP2D6 function and is the predominant functional variant in Caucasians, whereas *CYP2D6*10* reduces CYP2D6 function and is the predominant functional variant in Asians [78]. Studies in both Caucasian and Asian populations have yielded both protective (e.g., [63] and [57]) and causal (e.g., [72] and [53]) associations between CYP2D6 inhibition and breast cancer recurrence. While variation in the population distribution of the potency of inhibition of *CYP2D6* variants may contribute to variation in study results, this explanation would only hold if there is a true non-null association. Therefore, the pattern of clinical epidemiology results does not follow the pattern expected if population-dependent variability in potency was important.

As a second consideration, the quality of exposure data used to characterize CYP2D6 inhibition has varied widely. With regard to the studies of drug–drug interactions, sources of medication data include retrospective data from medical record review and prospective data from prescription claims databases. In this context, 'retrospective' indicates that data on SSRI prescription use were retrieved after follow-up data on recurrence were recorded, whereas 'prospective' indicates that the data on SSRI prescription use were recorded without the knowledge of the subject's recurrence status. This methodological distinction has important validity implications regarding the potential for differential misclassification bias of the SSRI exposure [79]. Nonetheless, studies of both designs have yielded null (e.g., [50] and [48]) and causal (e.g., [44] and [45]) estimates of the association between SSRI inhibition of CYP2D6 function and breast cancer recurrence.

With regard to the studies of *CYP2D6*–tamoxifen interaction, sources of genotyped DNA have included blood samples collected at diagnosis, archived tumor specimens and blood samples collected well after diagnosis (e.g., up to 11 years postdiagnosis [54]). When follow-up time begins at diagnosis, although blood samples were only collected well after diagnosis [53,54,58], a study is susceptible to immortal person-time bias. Another important consequence of the source of the DNA is the potential to comprehensively genotype the *CYP2D6* gene. DNA extracted from whole blood is of higher quality, allowing the use of more sophisticated and comprehensive genotyping, such as use of the AmpliChip® (a commercially available *CYP2D6* comprehensive genotyping tool [80]). DNA extracted from archived tumor or adjacent normal tissue is of lower quality, currently precluding the use of the AmpliChip. Comprehensive genotyping of the *CYP2D6* gene is important because *CYP2D6*4* is not the only variant that eliminates CYP2D6 function. Other alleles associated with no enzymatic activity include *CYP2D6*3* through **8, *11* through **16, *18* through **20, *38, *40, *42* and **44*. Similarly, *CYP2D6*10* is not the only variant that reduces *CYP2D6* function without eliminating it. Other alleles associated with reduced enzyme function include *CYP2D6*9, *10, *17, *29, *36, *37* and **41* [39].

The studies included in this article have varied widely in how comprehensively they genotyped *CYP2D6* (TABLE 1). A total of six studies genotyped only *CYP2D6*4* or only CYP2D6*10; while the others genotyped at least one other functional variant. In general, studies that more comprehensively genotyped the *CYP2D6* gene reported higher relative risks of breast cancer recurrence or breast cancer-specific mortality associated with *CYP2D6* inhibition. This pattern would be expected if failure to comprehensively genotype *CYP2D6* resulted in substantial nondifferential misclassification of the CYP2D6 functional phenotype, a phenomenon reported with empirical evidence in two studies [70,72]. In the Schroth study, approximately a third of the breast cancer patients were misclassified with regard to presumed CYP2D6 function when it was based on only the **4* mutation, compared with when it was based on comprehensive genotyping using the AmpliChip [72]. The relative risk associating breast cancer recurrence with poor CYP2D6 function increased from a nearly null association (1.3; 95% CI: 0.5–3.7) when based on only the **4* mutation to

a strong positive association (2.9; 95% CI: 1.4–6.1) when based on more than 30 mutations assayed by the AmpliChip.

The large cohort study of Abraham *et al.* [69], which genotyped the most prevalent *CYP2D6* functional alleles and yielded a null result, somewhat counters the results of these two studies. In addition, two studies nested within major adjuvant treatment trials and with broad – but not comprehensive – genotyping of *CYP2D6* were presented at the 2010 San Antonio Breast Cancer Symposium (TX, USA). A total of seven *CYP2D6* alleles were genotyped in the Arimidex, Tamoxifen, Alone or in Combination trial on 588 patients who received tamoxifen only and 615 patients who received anastrozole [65]. The Breast International Group (BIG 1–98) trial genotyped eight *CYP2D6* alleles in almost 5000 postmenopausal hormone-responsive breast cancer patients randomized to either letrozole or tamoxifen [64]. Both trials reported a near-null association between reduced *CYP2D6* function and breast cancer recurrence.

In our large population-based case-control study of polymorphisms in the *CYP2D6* gene and breast cancer recurrence [68], we implemented a quantitative bias analysis to account for the lack of comprehensive genotyping data (only *CYP2D6*4* was genotyped). In this analysis, we assumed that cases of recurrence were more likely to carry alleles with reduced-function than were controls. All of the parameters of the bias model were informed by published external data sources. Consistent with the Abraham study [69] and the studies presented at the 2010 San Antonio Breast Cancer Symposium [64,65], our bias analysis suggests that comprehensive genotyping of *CYP2D6* would have had little effect on the near-null results.

A third consideration is the potential for tamoxifen adherence to vary across studies and within categories of *CYP2D6* inhibition, which may partially explain the heterogeneity of reported associations. Approximately half of tamoxifen treated patients do not complete the intended duration of their tamoxifen therapy [81]. Failure to complete the intended course is related to recurrence risk [82], especially in conjunction with *CYP2D6* genotype [70], and in fact, may be caused by *CYP2D6* genotype [83]. If lack of adherence is caused by genotype and in turn causes recurrence, then adherence would be a causal intermediate between *CYP2D6* genotype and recurrence. Results adjusted for adherence would be more biased than without adjustment, usually towards the null [84]. Although adjustment for a causal intermediate is a well-known error in epidemiologic research [85], and we have made the argument earlier with specific regard to the association between *CYP2D6* inhibition and recurrence [8], reviews continue to erroneously raise failure to control for adherence as a problem in the body of literature on the topic [86].

## Evidence for other aspects of tamoxifen metabolism to modify its effectiveness

The polymorphic variants of CYP2D6 have been the most studied enzymes in tamoxifen's metabolic pathway, probably due to 4-hydroxy-*N*-desmethyl-tamoxifen binding to the ER with 100-fold higher affinity than tamoxifen and being present in the serum at higher concentrations than 4-hydroxytamoxifen. Nonetheless, as previously noted [8,26], with standard-dose regimens, tamoxifen and its metabolites are present at such abundant concentrations that they overwhelm the receptor. Thus, even among poor metabolizers (*CYP2D6*4/*4* and women simultaneously taking paroxetine), tamoxifen and its metabolites should still exert their anti-tumorigenic effects. Future perspectives on this topic might focus on the complete metabolic pathway, which might allow the identifcation of gene–gene interactions that sufficiently affect the profile of tamoxifen metabolites to have a clinical impact. In the only such comprehensive evaluation of the tamoxifen metabolic pathway to date [87], which was conducted in the prevention setting, *CYP2D6* variants were not strongly related to breast cancer occurrence. However, when the entire pathway was considered, *CYP2D6* was identified as a node that interacted with variant alleles in the other

tamoxifen-metabolizing genes. In the following section we briefly review candidate genes and the evidence suggesting that their variants might affect the profile of tamoxifen metabolites.

Cytochrome P450 2C19 plays a role in metabolizing tamoxifen into 4-hydroxytamoxifen and *N*-desmethyltamoxifen (FIGURE 1) [88,89]. Three polymorphic variants of *CYP2C19* have been identified (*CYP2C19*2, *3* and **17*). In contrast to *CYP2D6*4*, which eliminates enzymatic activity, the **17* variant of *CYP2C19* confers increased gene transcription and subsequently increased enzymatic activity [90]. The result could be an increase in the rate of tamoxifen activation in women carrying the allele. Gjerde *et al.* reported that increased CYP2C19 activity was associated with increased serum concentrations of 4-hydroxytamoxifen, and that CYP2D6 inhibition affects the ratio of 4-hydroxytamoxifen to tamoxifen, but not among individuals with the *CYP2C19*17* variant [88]. In addition to reporting higher rates of recurrence among women with genetic inhibition of CYP2D6, Schroth *et al.* found that carriers of the *C Y P2C19*17* variant had a lower risk of breast cancer recurrence, with the strongest effect seen in homozygotes [59]. Regarding the other *CYP2C19* variants, a study of Japanese breast cancer patients reported that *CYP2C19*2* and *CYP2C19*3* were not predictive indicators of response to tamoxifen treatment [57]. By contrast, a Dutch group reported that the *CYP2C19*2* variant predicted better survival in tamoxifen-treated breast cancer patients [91].

In the absence of functional CYP2D6, CYP2C9 takes the lead in catalyzing the formation of 4-hydroxy-*N*-desmethyl-tamoxifen, via initial 4-hydoxylation of tamoxifen [21,24] followed by demethylation catalyzed by CYP3A4 and CYP3A5 (FIGURE 1). Genetic variants of *CYP2C9* (*CYP2C9*2* and *CYP2C9*3*) reduce its catalytic ability and lower the production of 4-hydroxytamoxifen [24]. Thus a combination of lower CYP2D6 activity and reduced-function CYP2C9 may reduce an individual's ability to metabolize tamoxifen to 4-hydroxy-*N*-desmethyl-tamoxifen. However, combined inheritance of these nonfunctional or reduced-function alleles is likely to occur at a very low frequency.

While most research has focused on the metabolic enzymes involved in 4-hydroxylation of tamoxifen, which confers 100-fold greater binding affinity with the ER, demethylation is also an important step in the ultimate production of 4-hydroxy-*N*-desmethyl-tamoxifen. CYP3A4 and CYP3A5 primarily catalyze this metabolic step (FIGURE 1) [10]. *CYP3A4*1B* is a polymorphism in the untranslated region upstream of the DNA coding sequence, and thus affects mRNA expression levels. Although it is expected to have little functional consequence [92], carriers of this allele were at increased risk for recurrence in the only study that has evaluated it [67]. *CYP3A5*3*, which results in a truncated protein, affects the profile of tamoxifen metabolites [88], and has been related to improved disease-free survival in one study [62], marginally poorer survival in a second study [67] and had a null association in a third [93].

The secondary tamoxifen metabolites are sulfonated in a reaction catalyzed by sulfotransferases (especially SULT1A1) [94] or glucoronidated in a reaction catalyzed by UDP-glucoronosyltransferases (especially UGT1A8, UGT1A10, UGT2B7 and UGT2B15 ) [16]. Sulfonation and glucoronidation facilitate excretion by increasing the metabolite's water solubility and reduce the metabolite's activity because addition of the charged sulfonate or glucoronyl moiety prevents binding to the ER (FIGURE 1). Sulfonated tamoxifen metabolites are also desulfonated to their active forms by SULT1A1 and SULT1E1 (estrogen sulfotransferase; see FIGURE 1) [95].

Sulfotransferase 1A1 is polymorphic, with a wild-type allele (*SULT1A1*1*) and a variant allele (*SULT1A1*2*) [96,97]. The sulfotransferase produced by the variant allele has twofold

reduced activity [98] and reduced thermostability [99], which results in a lower enzyme concentration. Women with reduced SULT1A1 activity are expected to have higher concentrations of the secondary tamoxifen metabolites, because they cannot deactivate them as rapidly as women with the fully functional gene product. The profile of tamoxifen metabolite concentrations consistent with this expectation has been observed *in vivo* [14,15]. In three studies of breast cancer patients treated with tamoxifen, women with the *SULT1A1*2* variant had a higher hazard of recurrence than women with the wild-type allele in one study [56], a lower risk of recurrence in a second study [63] and approximately the same risk of recurrence in the third study [62].

Among the UGT enzymes that play a role in the detoxification of tamoxifen, *UGT1A10* has a polymorphic variant (*UGT1A10*$^{139\text{Lys}}$) [16], *UGT2B7* has a variant allele (*UGT2B7*2*) with a prevalence of approximately 50%, and both variant alleles confer reduced enzymatic activity [16,100]. *UGT2B15* has a variant allele (*UGT2B15*2* ) with at least a twofold higher rate of catalytic activity [101] . *UGT1A8* has two polymorphic variants (*UGT1A8*2* and *UGT1A8*3*) [16] – the *UGT1A8*2* variant has activity similar to the wild type, but the *UGT1A8*3* allele has significantly reduced activity compared with the wild type [16]. *UGT2B7* is thought to be the most active hepatic UGT enzyme with respect to tamoxifen metabolism [13]. Despite the high prevalence of some of the mutations (e.g., *UGT2B7*2*) and the compelling evidence for the role of UGTs in tamoxifen metabolism [13], the impact of inter-individual variation of these enzymes on tamoxifen metabolism and breast cancer recurrence has only been investigated in two published studies [56,62] and one conference abstract [66]. Women with the increased-function *UGT2B15*2* variant are expected to have lower concentrations of the secondary tamoxifen metabolites because they deactivate them more rapidly than women with the wild-type allele. Consistent with this expectation, two of the studies of breast cancer patients treated with tamoxifen reported that women with the variant allele had a higher rate of recurrence than women with the normal function allele, although the difference was imprecisely measured. By contrast, the conference abstract reported better survival among women with high UGT2B7 activity (the genotype associated with increased elimination of endoxifen, so expected to have poorer survival). More detailed examination of these findings awaits publication of the full report.

Taken together, these data illustrate the importance of considering the entire metabolic pathway for tamoxifen. Given the complexity of the metabolic pathway, it seems unlikely that the key to tamoxifen resistance lies within allelic variation of a single gene, even if that gene (*CYP2D6*) encodes a major enzyme in its metabolic pathway. Rather, the combined effect of variation in all enzymes involved in its metabolic pathway is likely to determine treatment effectiveness, if the metabolic profile has any impact at all. As demonstrated by the recent work of Dunn *et al.* [87], the combined effect of several changes in the function of metabolic enzymes within the entire tamoxifen metabolic pathway may be the key to evaluating its effectiveness.

## Expert commentary

Cytochrome P450 enzymes metabolize tamoxifen to more active intermediate metabolite forms [10], each with its own binding affinity to the ER. All of the enzymes in this pathway are polymorphic, and the changes in function related to genotypes contribute to interindividual differences in tamoxifen metabolite concentrations in the serum [14–16]. Most clinical epidemiology studies examining the associations between gene variants and breast cancer outcomes have focused on CYP2D6.

Epidemiologic studies associating reduced CYP2D6 function with risk of breast cancer recurrence have reported widely heterogeneous results, and no adequate explanation has

been proffered for this variability. We have previously addressed criticisms based on small sample size, survivor and other selection biases, potential for uncontrolled confounding by prognostic markers, and information bias arising from retrospective or absent information on use of CYP2D6-inhibiting medications or from noncentralized testing of ER expression [8]. In this article, we have also considered and rejected as complete explanations the variable potency of CYP2D6 inhibition, the variable quality of exposure information on CYP2D6 inhibition and failure to control for adherence to tamoxifen over the full duration of its intended course.

While the inconsistent pattern of associations remains, we note that the most recent large and high-quality studies have consistently reported near-null associations [48,64,65,68,69]. In addition, summary estimates of the association have consistently been near-null [8,102,103], and remain near-null in our quantitative meta-analyses.

The compelling molecular and pharmacologic hypotheses have prompted some to advocate the implementation of *CYP2D6* genotyping of breast cancer patients who are candidates for tamoxifen therapy in routine clinical practice. However, as our article and meta-analyses indicate, the results of clinical epidemiologic scientific studies present no sound foundation for this recommendation. Overall, there is little variation in the effectiveness of tamoxifen among individuals with varying degrees of CYP2D6 inhibition induced either by receipt of inhibiting medications or by functional polymorphisms in the *CYP2D6* gene. It is possible that the variations in the profile of metabolite concentrations associated with CYP2D6 inhibition are of no consequence with regard to breast cancer recurrence and survival. It is also possible that the focus on CYP2D6 inhibition has masked the complexity of the true underlying biology, which requires a more complete assessment of the function of many genes whose products metabolize tamoxifen.

## Five-year view

Although breast cancer prevention remains a considerable public health challenge, effective screening and ever-advancing therapies continue to improve the prognosis of breast cancer patients. More than 30 years ago, anti-estrogen therapy was understood to be most effective against tumors that expressed the ER. This result presaged the era of personalized medicine, and personalization of breast cancer therapies will probably continue to provide a model for individualized treatment regimens. With regard to the identification of breast cancer patients who are, or are not, good candidates for tamoxifen therapy by virtue of their capacity to metabolize the drugs, research will probably develop along one of three paths.

First, it is possible that metabolic capacity does affect tamoxifen's anti-estrogenic potency, but that the focus on CYP2D6 inhibition has told only a part of this story. Future research will have to investigate the complete metabolic pathway and multiple variant functional alleles in multiple metabolic enzymes. The complexity of modeling the complete pathway, and the requisite study size to evaluate combinations of genotypes that number in the tens of thousands, currently preclude such studies. However, *in vitro* models could be generated to incorporate combination profiles of these genetically variable metabolic enzymes. Such *in vitro* models could provide Bayesian priors, that is, a system to categorize breast cancer patients whose genotypes have similar metabolic profiles. These categorizations would reduce the requisite study size, and might allow a more complete evaluation of the association between metabolic capacity and tamoxifen effectiveness. The research model is very similar to the one already applied to CYP2D6 inhibition, in which observed variations in metabolic profile associated with *CYP2D6* genotype or CYP2D6-inhibiting medications provided a prior basis for evaluating the association between these characteristics and breast cancer outcomes in tamoxifen-treated breast cancer patients. The challenge in the coming

years will be to extend that model to multiple metabolic enzymes, each with functional variants that could affect the metabolic capacity to different extents.

Second, it is possible that CYP2D6 inhibition, or other modes of suboptimal tamoxifen metabolism, only conveys an increased risk of breast cancer in a subset of patients. Premenopausal women are a logical candidate subset because tamoxifen remains a guideline therapy only in these women. Furthermore, their higher endogenous concentration of estrogen suggests that impeded competition of tamoxifen metabolites for binding to the ER would be most relevant. No clinical epidemiology study has been restricted to premenopausal women or presented a subanalysis limited to premenopausal women. Wu *et al.* recently proposed that low concentrations of endoxifen may increase recurrence risk in tamoxifen-treated women but only among those whose tumors do not express ER-β [104]. This second subgroup, now demarcated by two biomarkers (genetic inhibition of *CYP2D6* and lack of ERβ expression), will require a very large study population in order to precisely estimate the three-way interaction between drug (tamoxifen), gene (*CYP2D6* genotype) and protein (ER-β expression).

Finally, it is possible that research focused on CYP2D6 inhibition has already told the complete story. These studies now appear now to be converging on a null or small association. Regardless of metabolic capacity, tamoxifen and its metabolites may be present at the site of action in sufficient concentrations to adequately antagonize growth stimulation by the ER. If that idea turns out to be correct, then it is likely that genotype-guided tamoxifen therapy will not come to fruition.

Key issues

- Tamoxifen is metabolized by several polymorphic enzymes from its administered form to more active metabolites. Genetic variation in these polymorphic enzymes contributes to interindividual differences in tamoxifen metabolite concentrations in the serum.
- The CYP2D6 enzyme is one of several enzymes catalyzing the formation of tamoxifen's metabolites. CYP2D6 has several polymorphic variants, which confer a fully functional, reduced-function or nonfunctional enzyme.
- The therapeutic dose of tamoxifen is set such that it and its metabolites overwhelm estrogen in competing for the estrogen receptor, depriving the breast tumor cell of estrogen-induced growth stimulation. A few-fold reduction in the concentration of one active metabolite, owing to genetic variation or drug-induced inhibition of CYP2D6, is unlikely to affect the ability of tamoxifen to block estrogen-induced growth stimulation.
- There is little evidence to suggest variation in the effectiveness of tamoxifen among individuals with varying degrees of CYP2D6 inhibition induced either by the receipt of inhibiting medications or by functional polymorphisms in the *CYP2D6* gene.
- Given that several enzymes play a role in the tamoxifen metabolic pathway, comprehensive genotyping of the *CYP2D6* gene and of genes encoding other enzymes involved in the entire tamoxifen metabolic pathway may be key to evaluating the genotype-guided effectiveness of tamoxifen.
- To date, the clinical epidemiology studies investigating the outcome of tamoxifen treatment in breast cancer patients have mainly focused on only one of these polymorphic enzymes, namely, cytochrome P450 2D6.

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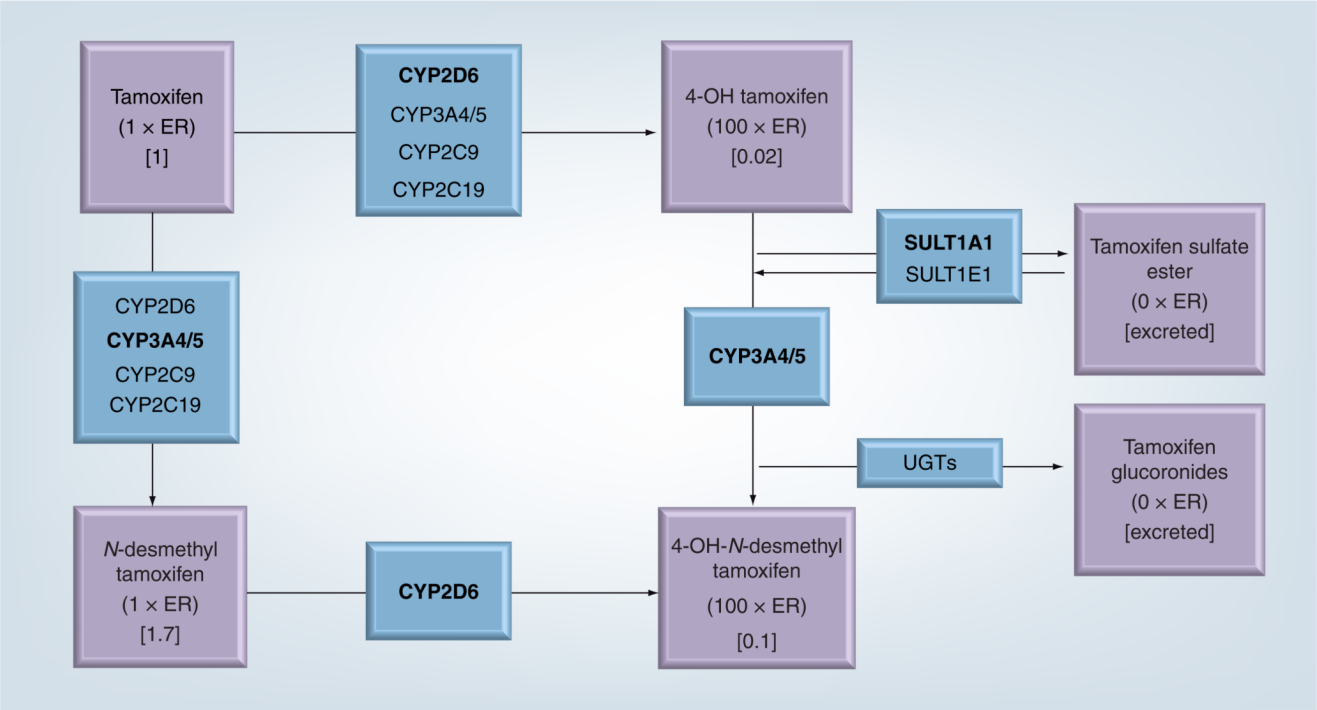


**Figure 1. Major metabolic pathways for tamoxifen**

Bold type denotes the enzyme(s) primarily involved in each step.

C: Plasma concentration of the metabolite, relative to tamoxifen's concentration, after 4 months of tamoxifen therapy at 20 mg/day; CYP: Cytochrome P450; N × ER: Binding affinity to estrogen receptor relative to tamoxifen itself; UGT: Uridine 5′-diphospho-glucuronosyltransferases.

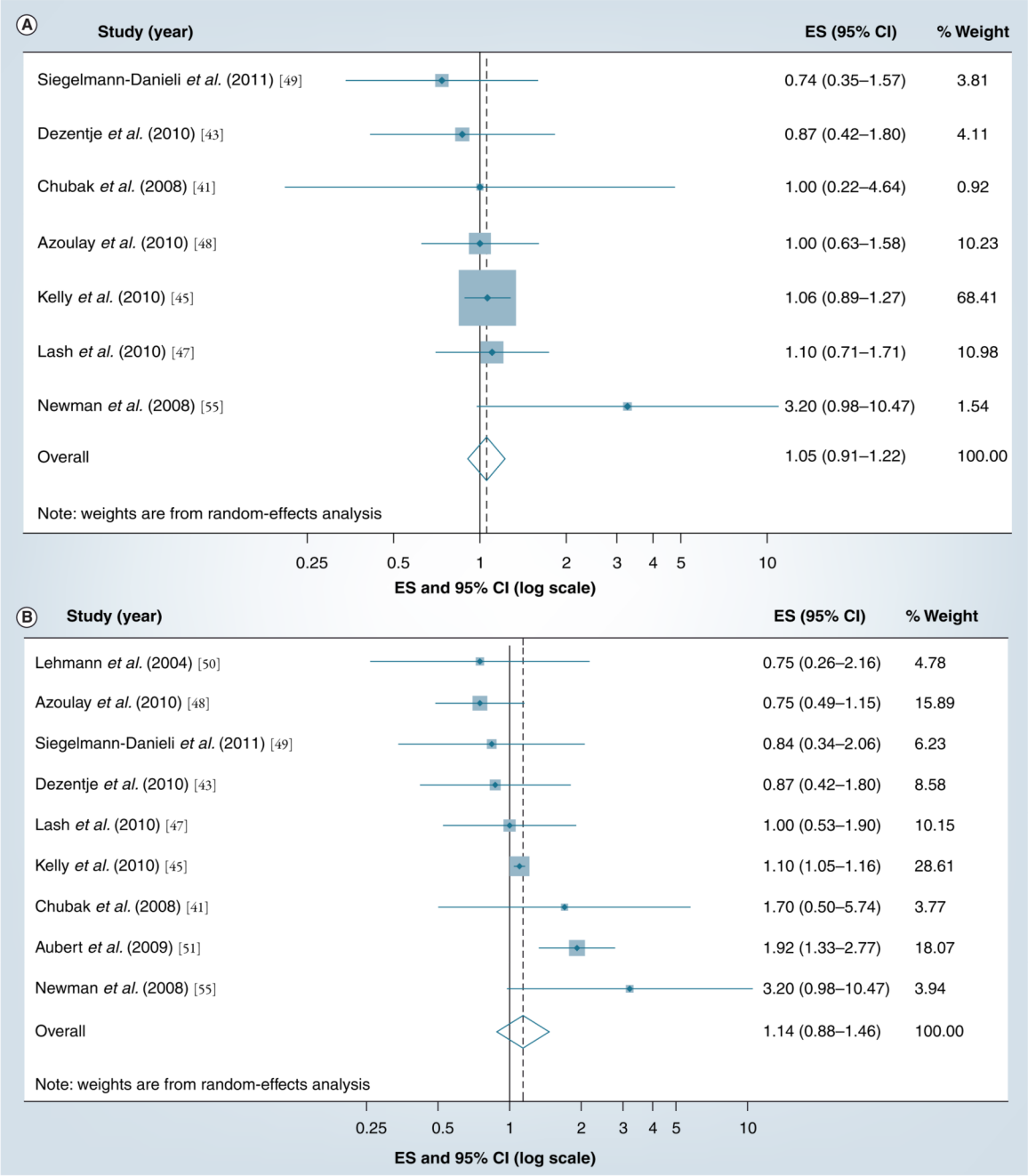


**Figure 2. The summary effect size and 95% confidence intervals for the association between concurrent use of (A) a weak CYP2D6 inhibitor drug and (B) a strong CYP2D6 inhibitor drug and breast cancer recurrence/survival**

Summary ES and 95% confidence intervals were estimated using a fixed-effects meta-analytical model. All statistical tests were two-sided. The size of each square is an illustrative representation of the study weight. The horizontal lines represent the CIs. The diamond represents the summary ES and 95% CIs.

CI: Confidence interval; ES: Effect size.

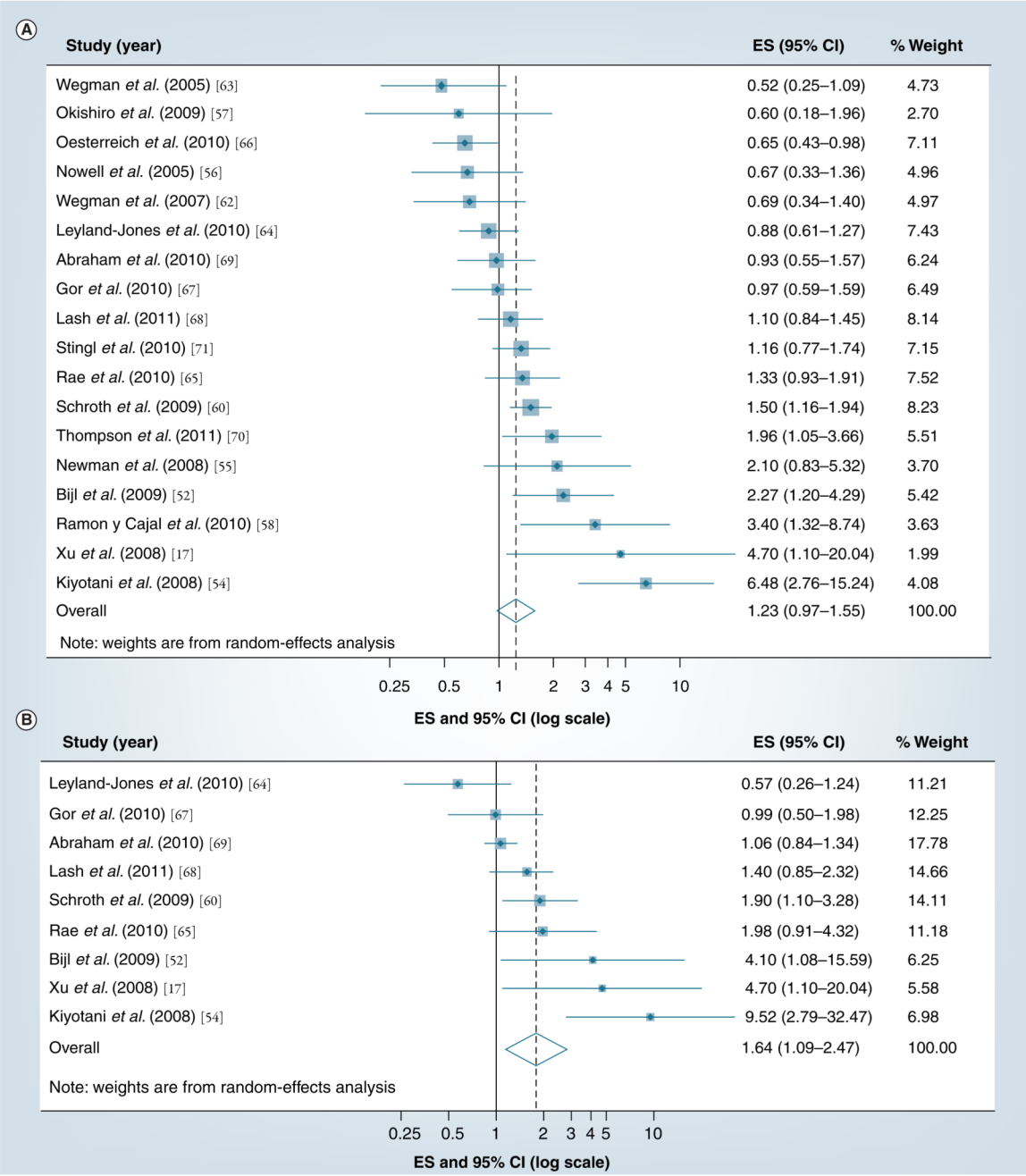


**Figure 3. The summary effect size and 95% confidence intervals for the association between (A) inheritance of any and (B) inheritance of two nonfunctional variants of *CYP2D6*4* or *CYP2D6*10* and breast cancer recurrence**

Summary ES and 95% CIs were estimated using random-effects meta-analytical models. All statistical tests were two-sided. The size of each square is an illustrative representation of the study weight. The horizontal lines represent the CIs. The diamond represents the summary ES and 95% CIs.

CI: Confidence interval; ES: Effect size.

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**Table 1**

List of studies associating *CYP2D6* inhibition by genotype with breast cancer recurrence.

| Study (year) | RR | *2 | *3 | *4 | *5 | *6 | *7 | *9 | *10 | *14 | *17 | *18 | *20 | *21 | *35 | *36 | *41 | UM | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Wegman *et al*. (2005) | 0.52 | | | X | | | | | | | | | | | | | | | [63] |
| Okishiro *et al*. (2009) | 0.60 | | | | | | | | X | | | | | | | | | | [57] |
| Nowell *et al*. (2005) | 0.67 | | X | X | | X | | | | | | | | | | | | | [56] |
| Wegman *et al*. (2007) | 0.69 | | | X | | | | | | | | | | | | | | | [62] |
| Leyland-Jones *et al*. (2010 ) | 0.88 | | X | X | | X | X | | X | | X | | | | | | X | | [64] |
| Gor *et al*. (2010) | 0.97 | | | X | | | | | | | | | | | | | | | [67] |
| Abraham *et al*. (2010) | 1.01 | | | X | X | X | | X | X | | | | | | | | X | X | [69] |
| Lash *et al*. (2011) | 1.1 0 | | | X | | | | | | | | | | | | | | | [68] |
| Stingl *et al*. (2010) | 1.16 | | | X | | | | | | | | | | | | | | | [71] |
| Rae *et al*. (2010) | 1.33 | X | X | X | | X | | | X | | X | | | | | | X | | [65] |
| Goetz *et al*. (2007) | 1.91 | | X | X | Y | | | | | | | | | | | | Y | | [44,60] |
| Thompson *et al*. (2011) | 1.96 | X | X | X | X | X | X | X | X | | | | | | X | | X | X | [70] |
| Newman *et al*. (2008) | 2 .1 0 | | X | X | X | | | | | | | | | | | | X | | [55] |
| Schroth *et al*. (2007) | 2.24 | Y | Y | X | X | Y | Y | Y | X | | | | | | Y | | X | | [59, 60] |
| Bijl *et al*. (2009) | 2.27 | | | X | | | | | | | | | | | | | | | [52] |
| Ramon y Cajal *et al*. (2010) | 3.40 | X | X | X | X | X | X | X | X | | | | X | | X | | X | X | [58] |
| Xu *et al*. (2008) | 4.70 | | | | | | | | X | | | | | | | | | | [17] |
| Kiyotani *et al*. (2008, 2010) | 6.65 | | | X | X | X | | | X | X | | | | X | | Y | X | Y | [53, 54] |

Studies are ranked from lowest RR point estimate to highest, and plotted against the *CYP2D6* alleles that were genotyped.

RR: Relative risk; UM: Ultrametabolizer; X: Genotyped; Y: Genotyped in an update of the same or an overlapping cohort.