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Genotype-guided tamoxifen therapy; time to pause for reflection?

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Abstract

Tamoxifen remains a cornerstone of adjuvant therapy for early stage breast cancer patients with estrogen receptor-positive tumors. Accurate markers of tamoxifen resistance would allow prediction of tamoxifen response and personalization of combined therapies. Recently, it has been suggested that patients with inherited nonfunctional alleles of the cytochrome P450 *CYP2D6* may be poor candidates for adjuvant tamoxifen therapy because women with these variant alleles have reduced concentrations of the tamoxifen metabolites that most strongly bind the estrogen receptor. In some studies, women with these alleles have a higher risk of recurrence than women with two functional alleles. However, dose-setting studies with clinical and biomarker outcomes, studies associating clinical outcomes with serum concentrations of tamoxifen and its metabolites, and a simple model of receptor binding, all suggest that tamoxifen and its metabolites should reach concentrations sufficient to achieve the therapeutic effect regardless of *CYP2D6* inhibition. The ten epidemiology studies of the association between *CYP2D6* genotype and breast cancer recurrence report widely heterogeneous results with relative risk estimates outside the range of reasonable bounds. None of the explanations proposed for the heterogeneity of results account adequately for the observed variability and no design feature sets apart any study or subset of studies as most likely to be accurate. The studies reporting a positive association may receive the most attention because they reported a result consistent with the profile of metabolite concentrations; not because they are more reliable by design. We argue that a recommendation for *CYP2D6* genotyping of candidates for tamoxifen therapy, and its implicit conclusion regarding the association between genotype and recurrence risk, is premature.

Introduction

Early stage breast cancer patients with estrogen receptor-positive tumors are candidates for adjuvant hormonal therapy, which nearly halves their recurrence risk (1). Current guidelines

recommend the following adjuvant hormonal therapies to reduce recurrence risk in breast cancer patients with estrogen receptor-positive disease (2–4). Premenopausal patients should receive tamoxifen, a selective estrogen receptor modulator, for five years. Postmenopausal patients should receive aromatase inhibitors (AIs) either as initial therapy or after treatment with tamoxifen. Postmenopausal women with contraindications to AIs or who decline AIs should receive five years of tamoxifen therapy. Thus, tamoxifen remains a cornerstone of adjuvant breast cancer therapy.

Tamoxifen and AIs prevent the estrogen receptor from promoting tumor cell growth by different mechanisms (5). AIs block the conversion of circulating adrenal androgens to estrogen by the enzyme aromatase in peripheral tissues (6), thus almost entirely removing the main source of estrogen production in postmenopausal women and leaving little or no hormone to stimulate the estrogen receptor. Tamoxifen's metabolites antagonize the effects of estrogen in breast tumors by competing with the hormone at receptor binding sites (7).

Mechanisms of resistance to tamoxifen therapy, and predictive markers of susceptibility to resistance, have been widely researched (8–10). Accurate markers would be clinically important, allowing prediction of tamoxifen response and personalization of combined therapies (11;12) (Figure 1). Recently, it has been suggested that estrogen receptor-positive breast cancer patients with inherited nonfunctional alleles of the cytochrome P450 *CYP2D6* may be poor candidates for adjuvant tamoxifen therapy (13–15). The hypothesis that *CYP2D6* inhibition may reduce the protection conferred by tamoxifen therapy rests on two lines of evidence (16). First, women with *CYP2D6* variant alleles that reduce the enzyme function, or who take *CYP2D6*-inhibiting drugs such as some selective serotonin reuptake inhibitors (SSRIs), have reduced concentrations of the tamoxifen metabolites that most strongly bind the estrogen receptor (17). Second, in some studies, women with *CYP2D6* variant alleles have a higher risk of recurrence than women with two functional alleles (13).

Our objective is to critically consider both the pharmacologic and epidemiologic evidence regarding the association between *CYP2D6* inhibition and risk of breast cancer recurrence. We ultimately argue that the recommendation for *CYP2D6* genotyping in breast cancer patients, and its implicit conclusion regarding the association, is premature.

The pharmacologic evidence

Tamoxifen requires activation to metabolites to function fully (18). Tamoxifen and its primary metabolite (N-desmethyl tamoxifen) are metabolized to 4-hydroxytamoxifen (7;18;19) or 4-hydroxy-N-desmethyltamoxifen (20) (sometimes called endoxifen (21;22)). These 4-hydroxylated metabolites bind the receptor more than 100-fold more readily than tamoxifen, so are the most important modulators of the estrogen receptor in the tamoxifen pathway (23). Genetic polymorphisms or drug-drug interactions that reduce the function of their catalytic enzymes may reduce tamoxifen's effectiveness.

CYP2D6 alone catalyzes activation of N-desmethyltamoxifen to 4-hydroxy-N-desmethyltamoxifen (24) and accounts for ~45% of the activation of tamoxifen to 4-hydroxytamoxifen (19). About ten *CYP2D6* alleles account for the variation in its ability to activate tamoxifen (19;24) by reducing or eliminating enzyme activity (25). Women with two functional alleles have higher steady-state concentrations of 4-hydroxytamoxifen (19;26) and 4-hydroxy-N-desmethyltamoxifen (17;24;26) than women with no functional alleles. Women with one functional allele have intermediate levels (26). Similarly, women with two functional alleles taking the potent *CYP2D6* inhibitor paroxetine (a SSRI) have lower concentrations of 4-hydroxy-N-desmethyltamoxifen (17;24). These data have led to the compelling hypothesis that tamoxifen's effectiveness may be reduced in women with *CYP2D6* variant alleles or who take other medications that inhibit *CYP2D6* function.

A more complete examination of the evidence, however, suggests that little effect of *CYP2D6* inhibition on recurrence risk might be expected. A wide inter-individual variability in steady-state concentrations of tamoxifen and its metabolites has been long recognized (27), yet these variable concentrations have not been associated with clinical outcomes (28; 29) and dose-setting studies have not suggested that higher doses of tamoxifen improve the average response (28;30). Tamoxifen doses as low as 1 mg/day affect the cancer proliferation antigen Ki-67 equivalently to the typical dose of 20 mg/day (31;32).

Tamoxifen and its metabolites act by competing with estrogen for binding to the estrogen receptor. The key mechanistic question, therefore, is whether the reduced serum concentrations of active tamoxifen metabolites induced by *CYP2D6* inhibition substantially reduces binding of tamoxifen and its metabolites to the estrogen receptor. To answer, we use a simple model of estrogen receptor binding (33) that estimated that tamoxifen and its metabolites bind to more than 99% of estrogen receptors in postmenopausal women on a daily dose of 20 mg tamoxifen. Table 1 replicates the calculation using plasma concentrations of tamoxifen and its metabolites within strata of women with two functional *CYP2D6* alleles, one functional *CYP2D6* allele, or two nonfunctional *CYP2D6* *4 alleles (17). According to this model, there would be little difference in estrogen receptor binding by tamoxifen and its metabolites within women grouped by their *CYP2D6* genotype. The model yields similar results when using (a) serum concentrations in breast cancer patients with two functional *CYP2D6* alleles and grouped by whether or not they were taking paroxetine (17), (b) an estradiol concentration more typical of premenopausal women (275 pM, see Table 1), (c) a relative binding affinity for the 4-hydroxylated metabolites equal to the relative binding affinity of estradiol, or (d) the concentrations of 4-hydroxytamoxifen and 4-hydroxy-N-desmethyltamoxifen reported by Lim *et al* (34) within strata of women with no, one, or two *10 alleles of *CYP2D6*.

Like any model, this one simplifies a complex reality to emphasize a critical component of that reality. In this case, the model suggests that tamoxifen and its metabolites overwhelm the receptor — which is the therapeutic intent of this selective estrogen receptor modulator — and that the reported changes in the concentrations of the metabolites are unlikely to change that condition. If so, then the concentrations of tamoxifen and its metabolites should be sufficient to manifest fully tamoxifen's antitumorigenic effect regardless of whether *CYP2D6* inhibition reduces the concentration of some tamoxifen metabolites. Nonetheless, we recognize that this model has important limitations. For example, it takes no account of the high proportion of serum tamoxifen that is bound to protein (35) or competition with the estrogen receptor for tamoxifen and its metabolites by antiestrogen binding sites that interfere with different cytostatic mechanisms (36). Furthermore, intra-tumor concentrations of tamoxifen and its metabolites are usually higher than serum concentrations (32;37) and intra-tumor estrogen concentrations are not significantly different between pre- and post-menopausal women (38). Finally, different tamoxifen metabolites may modulate the estrogen receptor differently (39; 40), although under the model more than two-thirds of the binding activity derives from the two 4-hydroxylated metabolites and *in vitro* these metabolites have similar antiestrogenic potency (23), antiproliferative activity (24), and effects on global gene expression patterns (41).

The epidemiologic evidence

Search strategy and selection criteria

We searched the US National Library of Medicine's PubMed database with the keywords "tamoxifen" and "*CYP2D6*." We selected all papers published through 1 March 2009 regarding the association between *CYP2D6* gene variants and the risk of recurrence among women treated with tamoxifen for adjuvant breast cancer therapy. We also examined citations within the selected publications and within other publications on this topic.

Ten epidemiologic studies of the association between inheriting a variant *CYP2D6* allele and breast cancer recurrence risk have been published (42–51). An additional study has appeared only as an abstract and apparently used a cross-sectional design to study the association in a survivor population (52). An association in the prevention setting has been presented only as an abstract and letter (53;54). We do not discuss this association further because we lack complete information on the methods and results. Figure 2 depicts the ten published studies in the adjuvant setting and aspects of our epidemiologic perspective. To construct the figure (which is adapted from an earlier version (55)), we ranked the ten estimates of relative risk of recurrence from lowest to highest (citations (42) to (51) in ascending order). We then plotted each study's relative risk estimate and its 95% confidence interval (CI) against the inverse normal of its rank percentile (56). The integer above each study's error bar shows the number of women in the study with a recurrence and the variant allele(s). Because both recurrence and the variant allele are rare, this number primarily determines the precision of the relative risk estimates. The dashed horizontal lines depict reasonable upper-bounds on the relative risk one would expect to be observed in the studies plotted within their regions. We begin our epidemiologic perspective with the derivation of these bounds (see also the Appendix).

Bounds on the relative risk associating variant alleles with recurrence risk

To derive bounds, we set $R_{AI}/R_{Tamoxifen} = 0.85$ (95% CI=0.78, 0.93), where R_i is the recurrence risk in estrogen-receptor positive women treated with $i=AI$ or $i=tamoxifen$ and the estimate is pooled over two trials of initial treatment for five years (57). We set $R_{Tamoxifen}/R_{Placebo} = 0.59$ (95% CI=0.56, 0.63) where the estimate is from the overview of initial treatment with tamoxifen for 5 years versus a placebo (58). We did not account for random error about these summaries of trial evidence; their variances are much smaller than the variance of the estimate in any of the ten studies.

We then assumed that tamoxifen cannot be more effective than AIs in women with two functional alleles. This assumption presumes that estrogen-receptor binding by tamoxifen and its metabolites in women with two functional alleles cannot be more effective than the near absence of estrogen binding with its receptor in postmenopausal women on AI therapy. We also assumed that tamoxifen cannot be less effective than placebo in women with no functional allele. This assumption presumes that complete absence of *CYP2D6* activity cannot render tamoxifen therapy less effective than no hormonal therapy.

All ten studies of *CYP2D6* modification of tamoxifen effectiveness combined heterozygotes with either the homozygotes with no functional allele or with the homozygotes with two functional alleles. Five studies of *CYP2D6* *4 ((42) and (44) to (48)) used the former combination and the three studies of *CYP2D6* *10 ((43), (50) and (51)) used the latter combination. The five studies reporting a relative risk associating the *CYP2D6* *4 genotype with recurrence risk all estimated β , and the studies reporting a relative risk associating the *CYP2D6* *10 genotype with recurrence risk all estimated α , where β and α are the ratios of genotype-specific recurrence risks weighted by the prevalences of the genotypes. Using the algebraic derivation in the Appendix and the prevalence of *4 and *10 genotypes reported for Caucasian and Asian populations (59), respectively, we calculated $\beta \leq 1.55$ and $\alpha \leq 2$, which are the upper bounds on the relative risk depicted by the dashed horizontal lines in Figure 2. If one considers these bounds and the null as, respectively, an upper-limit and a lower-limit on the true relative risk, then none of the studies' point-estimates of relative risk fall within the bounds that pertain to their allele-specific result (although all of their intervals overlap the bounded region).

Review of the epidemiologic evidence

Summary—Figure 2 shows that the reported relative risks associating *CYP2D6* variant genotypes with breast cancer recurrence are highly heterogeneous (p for test of homogeneity <0.001). The pattern of heterogeneous estimates of relative risk, all outside the range of reasonable bounds and each relying on a small number of exposed recurrent cases, should alone caution against any causal inference.

The study by Goetz *et al.* (46) has received the most attention in many discussions of this topic (4;10;57;60–63). It was the only study cited in several reviews (4;10;57;63) and the only one used to inform a modeling exercise (64). The non-positive studies' results, when they are cited, are often attributed to confounding or selection bias (60), labeled as conflicting (61) or discordant (62), or characterized as having failed to support the theory (65).

Table 2 summarizes the design and analysis of each of the ten studies. There is no characteristic, or pattern of characteristics, that explains the heterogeneity of results. Many of the criticisms of the design and analysis of the studies that do not report a positive association are unfounded, or apply in equal measure to the studies that do report a positive association, as we will discuss below.

Heterogeneity arising from study designs—The study with the highest reported relative risk (51) included only women who completed five-years of tamoxifen therapy and survived to donate blood for genotyping, yet all of their person-time beginning at diagnosis was included in the analysis. Outcomes and person-time that occur before the last event required for participation in a study should be excluded from analysis to avoid bias (66). This study's cross-sectional design most likely introduced a selection bias, which can create the appearance of an association when none exists (67). The study with the third highest association (49), as well as a study by Lim *et al.* (34) that focused on metastatic disease, were also susceptible to the same design problem. Even with these studies eliminated from consideration, the relative risks reported in the remaining seven studies are substantially heterogeneous (p for test of homogeneity = 0.003).

A recent letter (16) emphasized the results of the Goetz study (46) because it was the only one that “derived from a prospective clinical trial” and because “the primacy of randomized, prospectively controlled trial data” should be taken into account. However, the entire study population in the first Wegman *et al* study (42), and more than one-third of the tamoxifen-exposed population in the second Wegman *et al* study (45), also derived from clinical trials. More importantly, no woman in any of the studies was randomized to her genotype by a human protocol. The women were randomized to treatments that involved tamoxifen, which provides no protection against confounding of the association between genotype and recurrence risk. Natural randomization of genotype (*i.e.*, Mendelian randomization) may yield no association between genotype and prognostic factors affecting recurrence risk, which would provide protection against confounding regardless of the source of information used to identify tamoxifen-treated breast cancer patients (*i.e.*, trial roster, clinical series, or registry).

In addition, none of the ten studies used prospective data collection. In each study, genotypes were assayed after the recurrences had occurred and, thus, they all have a retrospective design with respect to collection of genotype information (68). Presumably the researchers who assayed genotype were blinded to recurrence information, which protects against bias. Goetz (46) and Newman (47) included in their exposure definitions information on use of *CYP2D6* inhibiting medications while taking tamoxifen. This medication history was abstracted from medical records after the recurrences had occurred. Recurrences may have been documented in the same medical records, leaving open the possibility that abstractors collecting medication history (the exposure information) were not blinded to outcome information.

Heterogeneity arising from selection bias—A common criticism of the studies that did not report a positive association is the use of retrospectively assembled cohorts biased toward patients with available tumor specimens (61), which is a concern about selection bias. Selection bias arises when the exposure (*CYP2D6* genotype) and the outcome (breast cancer recurrence) both affect the probability of inclusion in the study population (67). Table 2 shows that each of the studies implemented some selection strategy, but the selection forces (often availability of tumor specimens) were unlikely to depend on *CYP2D6* genotype. They are therefore unlikely to have biased the estimate of association between *CYP2D6* genotype and recurrence risk in any study. Selection bias is an unlikely explanation for the heterogeneity of results. Selection of a survivor population in some studies (34;45;49;51) is much more likely to have biased these studies' estimates of association (69).

Heterogeneity arising from uncontrolled confounders—A confounder must be a cause of the outcome or a surrogate for such a cause. It must also be a cause of the exposure or must share common causes with the exposure, and must not be a causal intermediate between the exposure and the outcome (67). These principles inform our discussion of the potential for uncontrolled confounders to explain the heterogeneity of results.

Failure to adjust for adherence to the intended dose and duration of tamoxifen therapy has been proposed as one potential uncontrolled confounder (16;61). Nearly half of postmenopausal women do not complete the recommended five-year course of tamoxifen therapy (70). Only the second Wegman *et al* study adjusted for duration of completed tamoxifen therapy (45). If *CYP2D6* genotype is associated with adherence (71), and failure to adhere to the intended protocol increases the risk of recurrence, then adherence to the intended protocol would be a causal intermediate between *CYP2D6* genotype and recurrence. Results adjusted for adherence would be more biased (often, but not always, toward the null (72)), rather than less biased.

Similarly, failure to account for use of other *CYP2D6* inhibiting medications has been proposed as a potential unmeasured or uncontrolled confounder (61). Table 2 shows that three of the studies reporting a positive association accounted for use of other *CYP2D6* inhibiting medications (46;47;51) and three did not (48–50). More importantly, *CYP2D6* genotype is not likely to be associated with receipt of a *CYP2D6* inhibiting medication because this genotype would be unknown to the patient and provider at the time of the first prescription. Such a prescription cannot cause assignment to *CYP2D6* genotype and is unlikely to share a common causal ancestor, so prescription of a *CYP2D6* inhibiting medication does not satisfy the requisite causal structure of a confounder. *CYP2D6* genotype may affect adherence to *CYP2D6* inhibiting medications, although in the only randomized trial, *CYP2D6* genotype was not related to either the occurrence of adverse events or to adherence to paroxetine prescription (73). If genotype does affect adherence to other *CYP2D6* inhibitors, and use of those inhibitors affects recurrence risk, then this adherence would be a causal intermediate, so would not be a candidate for adjustment in any event.

Finally, prognostic markers — especially intended tamoxifen dose and duration (as opposed to adherence to the intended protocol), tumor stage, and receipt of other treatments — have been proposed as uncontrolled confounders (13;61;65). None of these variables is likely caused by *CYP2D6* genotype or to share a common causal ancestor with *CYP2D6* genotype. They do not satisfy the requisite causal structure of a confounder. In fact, many of the studies noted that tumor prognostic markers were not associated with *CYP2D6* genotype. Adjustment for such variables may introduce bias or reduce the precision of the estimate of association. For example, Xu *et al.* adjusted for age, tumor size, lymph node status, clinical stage, C-erbB2 status, estrogen receptor or progesterone receptor status, surgery, and adjuvant treatment (50). With only fifteen recurrences (and only 4 in the reference genotype category), adjustment for nine variables (each with at least two categories) creates an analysis likely to yield an association inflated by sparse

data bias (74). Indeed, the crude association imputed from the survival curves equals a rate ratio of 3.4 associating *CYP2D6* *10/*10 genotype with breast cancer recurrence, compared with *CYP2D6* */*10 or */*. Advanced stage and estrogen receptor-negative tumors were more prevalent in the women with *10/*10 genotype, so adjustment should have reduced the estimate of association toward the null. Instead, adjustment yielded a higher rate ratio with a very wide interval (4.7, 95% CI 1.1, 20). This type of analytic incongruence is a hallmark of sparse data bias.

All ten studies included information on the intended dose and duration of tamoxifen therapy (Table 2) which, although perhaps suboptimal, should have conferred some protection against recurrence in the study populations (75). If *CYP2D6* inhibition reduces that protective effect, then the studies should have detected the reduction. In some studies, more than one tamoxifen regimen was included. However, *CYP2D6* genotype could not be caused by the prescribed tamoxifen regimen, nor is it likely that they share a common causal ancestor. Tamoxifen dose regimen does not, therefore, satisfy the requisite causal structure of a confounder.

We conclude that there is no uncontrolled confounder that accounts for the heterogeneity of the study results.

Heterogeneity arising from information bias (misclassification)—Goetz *et al* (61) suggested that non-centralized testing of estrogen receptor expression, resulting in the inclusion of ER-negative patients, may explain why some studies did not report a positive association. Centralized testing is unlikely to improve the positive predictive value compared with routine pathology laboratory analysis. In one validation study, a positive estrogen-receptor assay in a pathology report had a positive predictive value of 93% when a central laboratory immunohistochemistry assay was used as the gold-standard (76). A second validation study showed similar agreement between pathology laboratory assays and centralized testing (77). Few subjects characterized as estrogen receptor-positive in studies without centralized testing of estrogen receptor expression are likely to be false-positives, and these errors are unlikely to depend on subjects' *CYP2D6* genotype. The trial protocol in the Goetz *et al* study (46) allowed estrogen receptor expression to be assayed by either biochemistry or immunohistochemistry (78), which do have different positive predictive values (76). This allowance suggests that the Goetz *et al* study is also susceptible to variable errors in classification of estrogen receptor expression.

Variable rates of misclassification of estrogen receptor status is an unlikely explanation for the heterogeneity of the study results. A different sort of misclassification, however, likely affected the results of the study with the third highest association (49). In this study, subjects with similar *CYP2D6* phenotypes were classified into different exposure groups, apparently in an effort to maximize the strength of association. Such differential misclassification is likely to yield a strong bias away from the null.

Summary of potential sources of heterogeneous results—None of the explanations proposed for the widely heterogeneous results of the ten published studies account adequately for the observed variability. Five of the six studies that reported a positive association are susceptible to biases that might have led to overestimates of the association. Medical record abstractors in the Goetz (46) and Newman (47) studies may not have been blinded to subjects' recurrence history. The Xu *et al* study (50) was susceptible to sparse data bias; an adjustment that should have reduced its estimate of the association actually led to an increase, consistent with an effect of this bias. The Kiyotani *et al* and Ramon *et al* studies (49;51) used a cross-sectional design subject to survivor bias, and the Ramon *et al* study (49) was susceptible to differential misclassification. The only substantial bias apparent in the four studies that did not report a positive association between *CYP2D6* inhibition and recurrence risk is the inclusion

of immortal person-time and adjustment for tamoxifen adherence in the second Wegman *et al* study (45).

Conclusion

The hypothesis that inhibition of *CYP2D6* activity reduces the protection against breast cancer recurrence conferred by tamoxifen therapy rests on the observation that women carrying the variant allele or taking *CYP2D6* inhibiting drugs have lower serum concentrations of active metabolites (17;24;34). Dose-setting studies with clinical and biomarker outcomes, studies associating clinical outcomes with serum concentrations of tamoxifen and its metabolites, and a simple model of receptor binding, all suggest that tamoxifen and its metabolites should reach concentrations sufficient to achieve the therapeutic effect regardless of *CYP2D6* inhibition. Thus, to support the hypothesis, mechanistic data are needed to show how the change in metabolite concentration profile associated with inheriting the variant alleles reduces the protection against recurrence conferred by tamoxifen therapy, despite this evidence to the contrary. Initial *in-vitro* work relating receptor degradation to 4-hydroxy-N-desmethyltamoxifen concentration may provide such a mechanism (40). The potential for rebound and stabilization of receptor concentrations with longer incubation periods (79), and the potential for the study's pre-incubation protocol to have interfered with stabilization by chaperone proteins (80), both merit investigation.

None of the epidemiologic studies associating *CYP2D6* inhibition with a reduction in the protection against breast cancer recurrence conferred by tamoxifen therapy has reported an association within the bounds plotted in Figure 2. Associations outside these bounds imply that *CYP2D6* inhibition improves the effectiveness of tamoxifen therapy (associations <1), that tamoxifen therapy is more effective than AI therapy in women with two functional alleles, or that tamoxifen therapy is less effective than placebo in women with no functional allele. To support the hypothesis, the association between *CYP2D6* inhibition and recurrence risk must be within these bounds, or mechanistic data should be obtained to show how tamoxifen therapy can be more effective than an AI (in the first case) or less effective than placebo (in the second case).

Finally, neither the emphasis received by the Goetz *et al* study, nor the methodologic criticisms directed at the studies reporting associations below the null, are justified. The reported associations are widely heterogeneous and there is no design feature that sets apart any subset of studies. The studies reporting a positive association may have received the most attention because they reported the expected "right" answer. That is, the associations they report suggest that *CYP2D6* inhibition reduces the protection against recurrence conferred by tamoxifen therapy, a result consistent with the profile of metabolite concentrations. If the initial hypothesis had taken account of the dose-setting studies and overwhelming receptor binding by the metabolites of this selective estrogen receptor modulator, then the results of these studies may have been viewed differently.

The medical literature contains many errors in causal inference that can be traced to the combination of a seductive biologic mechanism and selective contemplation of epidemiologic research. The state of the science examining the association between *CYP2D6* inhibition and tamoxifen effectiveness is, at present, ripe for just such an error (81). More evidence should be collected before any conclusion is reached on the question of whether tamoxifen treatment in breast cancer patients should be based on the results of *CYP2D6* genotyping.

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Appendix

The studies reporting a relative risk associating the *CYP2D6* *4 genotype with recurrence risk estimated β , and the studies reporting a relative risk associating the *CYP2D6* *10 genotype with recurrence risk estimated α , where β and α are the ratios of genotype-specific recurrence risks weighted by the prevalence of the genotypes.

$$\beta = \frac{\frac{p_{\bar{a}\bar{a}}R_{\bar{a}\bar{a}} + p_{aa}R_{aa}}{p_{\bar{a}\bar{a}} + p_{aa}}}{R_{\bar{a}\bar{a}}}$$

and

$$\alpha = \frac{R_{aa}}{\frac{p_{\bar{a}\bar{a}}R_{\bar{a}\bar{a}} + p_{aa}R_{aa}}{p_{\bar{a}\bar{a}} + p_{aa}}}$$

In these equations, “p***” is the prevalence of the genotype denoted by the subscript and “R***” is the risk of recurrence among women with the genotype denoted by the subscript. We rearrange these equations for convenience as follows:

$$p_{\bar{a}\bar{a}}R_{\bar{a}\bar{a}} + p_{aa}R_{aa} = \beta R_{\bar{a}\bar{a}}(p_{\bar{a}\bar{a}} + p_{aa})$$

and

$$p_{\bar{a}a}R_{\bar{a}a} + p_{\bar{a}\bar{a}}R_{\bar{a}\bar{a}} = \frac{R_{aa}}{\alpha}(p_{\bar{a}a} + p_{\bar{a}\bar{a}})$$

To derive bounds, we begin with the assumption that tamoxifen cannot be more effective than AI in women with $\bar{a}a$ genotype. That is,

$$R_T = p_{\bar{a}\bar{a}}R_{\bar{a}\bar{a}} + p_{\bar{a}a}R_{\bar{a}a} + p_{aa}R_{aa}$$

$$0.85 = \frac{R_{AI}}{R_T} \text{ and } R_{AI} \leq R_{\bar{a}\bar{a}}$$

so

$$0.85 = \frac{R_{AI}}{R_T} \leq \frac{R_{\bar{a}\bar{a}}}{p_{\bar{a}\bar{a}}R_{\bar{a}\bar{a}} + p_{\bar{a}a}R_{\bar{a}a} + p_{aa}R_{aa}}$$

Subscript “T” denotes tamoxifen treatment and subscript “AI” denotes aromatase inhibitor treatment. The estimate of $0.85 = R_{AI}/R_T$ (95% CI=0.78, 0.93) is the pooled estimate of efficacy results from two trials of initial treatment with AI for 5 years versus an AI for five years (57).

We also assume that tamoxifen cannot be less effective than placebo in women with aa genotype. That is,

$$R_T = p_{\bar{a}\bar{a}}R_{\bar{a}\bar{a}} + p_{\bar{a}a}R_{\bar{a}a} + p_{aa}R_{aa}$$

$$0.59 = \frac{R_T}{R_p} \text{ and } R_{aa} \leq R_p$$

so

$$0.59 = \frac{R_T}{R_p} \leq \frac{p_{\bar{a}\bar{a}}R_{\bar{a}\bar{a}} + p_{\bar{a}a}R_{\bar{a}a} + p_{aa}R_{aa}}{R_{aa}}$$

Subscript “P” denotes placebo treatment. The estimate of $0.59 = R_T/R_P$ (95% CI=0.56, 0.63) is the summarized estimate of initial treatment with tamoxifen for 5 years versus a placebo from the most recent overview of the tamoxifen trials (82).

Substituting the respective equations rearranged for convenience into the denominator of the last equation of the first set of equations and into the numerator of the last equation of the second set of equations yields

$$0.85 \leq \frac{R_{\bar{a}\bar{a}}}{p_{\bar{a}\bar{a}}R_{\bar{a}\bar{a}} + R_{\bar{a}\bar{a}}\beta(p_{\bar{a}a} + p_{aa})}$$

and

$$0.59 \leq \frac{\frac{R_{aa}}{\alpha}(p_{aa} + p_{aa}^-) + p_{aa}R_{aa}}{R_{aa}}$$

Canceling R_{aa} in the first equation and R_{aa} in the second equation, then solving for β and α , respectively, we have

$$\beta \leq \frac{\frac{1}{0.85} - p_{aa}}{p_{aa} + p_{aa}^-}$$

and

$$\alpha \leq \frac{p_{aa} + p_{aa}^-}{0.59 - p_{aa}}$$

The prevalence of *4 and *10 genotypes reported for Caucasian and Asian populations (59), respectively, are

	*4 (Caucasians)	*10 (Asians)
p_{aa}	0.68	0.31
p_a	0.27	0.51
p_{aa}	0.05	0.18

Substituting the *4 prevalence values into the first equation and the *10 prevalence values into the second equation yields $\beta \leq 1.55$ and $\alpha \leq 2$, which are the bounds on the relative risk depicted by the dashed horizontal lines in Figure 2.

Figure 1.
A woman and her physician contemplate genotype-guided tamoxifen therapy.

Figure 2.

Ten studies of the *CYP2D6* association cited in ascending order ((42) to (51)). Error bars are 95% CI, integers are number of variant recurrences, and dashed lines are derived bounds.

Estimation of estrogen receptor (ER) binding by tamoxifen and some of its metabolites in postmenopausal and premenopausal women at steady state concentrations with treatment of 20 mg/day tamoxifen, stratified by *CYP2D6* *4 genotype. See the text for a discussion of limitations that pertain to this model.

Table 1

Postmenopausal women	relative binding affinity	2 functional concentration (pM)	<i>CYP2D6</i> alleles activity	1 functional concentration (pM)	<i>CYP2D6</i> allele activity	no functional concentration (pM)	<i>CYP2D6</i> allele activity
estradiol	100.0	25	2,500	25	2,500	25	2,500
tamoxifen	2.8	372,500	1,043,000	353,300	989,240	288,900	808,920
N-desmethyltamoxifen	2.4	653,400	1,568,160	687,300	1,649,520	664,100	1,593,840
4-hydroxytamoxifen	181.0	9,500	1,719,500	8,300	1,502,300	7,100	1,285,100
4-hydroxy-N-desmethyltamoxifen	181.0	78,000	14,118,000	43,100	7,801,100	20,000	3,620,000
ER binding by tamoxifen and metabolites			99.99%		99.98%		99.97%
Premenopausal women	relative binding affinity	2 functional concentration (pM)	<i>CYP2D6</i> alleles Activity	1 functional concentration (pM)	<i>CYP2D6</i> allele activity	no functional concentration (pM)	<i>CYP2D6</i> allele activity
estradiol	100.0	275	27,500	275	27,500	275	27,500
tamoxifen	2.8	372,500	1,043,000	353,300	989,240	288,900	808,920
N-desmethyltamoxifen	2.4	653,400	1,568,160	687,300	1,649,520	664,100	1,593,840
4-hydroxytamoxifen	181.0	9,500	1,719,500	8,300	1,502,300	7,100	1,285,100
4-hydroxy-N-desmethyltamoxifen	181.0	78,000	14,118,000	43,100	7,801,100	20,000	3,620,000
ER binding by tamoxifen and metabolites			99.85%		99.77%		99.63%

Table 2

Summary of study characteristics proposed as explanations for the observed heterogeneity of results

	Wegman (42)	Okishiro (43)	Nowell (44)	Wegman (45)	Goetz (46)	Newman (47)	Schroth (48)	Ramon (49)	Xu (50)	Kiyotani (51)
Design	retrospective	retrospective	retrospective	retrospective	retrospective	retrospective	retrospective	retrospective	retrospective	retrospective
Source population	trial, 1976+ by trial protocol	clinical series, 1998–2004 by pathology laboratory protocol	clinical series, 1985–1996 by pathology laboratory protocol	regional registry, 1986–1997; 35% in a trial by pathology laboratory protocol	trial, 1991–1995 trial protocol, biochemical or IHC	registry of BRCA1/2 carriers, unknown years by pathology laboratory protocol	clinical series, 1986–2000 by pathology laboratory protocol	clinical series, 1996–1998 by pathology laboratory protocol	clinical series, 1994–2005 by pathology laboratory protocol	clinical series, 1986–2006 by pathology laboratory protocol, centrally retested in 83%
ER testing										
Tamoxifen dose	40 mg/d; 2 years	20 mg/d; median 52 months (range 9–60 months)	not reported	40 mg/d for 2 or 5 years; 20 mg/d for 5 years	20 mg/d for 5 years	20 mg/d; median duration > 4 years	not reported	not reported	20 mg/d for 5 years	20 mg/d for 5 years
Follow-up duration	median 10.7 yrs	median 4.7 years	average 4.7 yrs	median 7.1 yrs	<11.4 yrs	median 10 years	median 6.4 yrs	mean 9 yrs	median 5.3 yrs	median 8 yrs
Age group	post-menopausal	78% post-menopausal	60% >50 yrs	post-menopausal	post-menopausal	27 to 68	median 68.4 yrs	40% post-menopausal	75% >50 yrs	median 50 yrs
Selection of study subjects	fresh frozen tumors (33%); ER+ (70%)	consecutive patients with archived blood samples	archived blocks (~64%)	restricted to cases surviving at least 2 years	available tissue (87%); successful genotyping (85%)	complete clinical data and treated with tamoxifen (27%); available genotype (92%)	available follow-up information and tumor specimen	outpatient follow-up care in 2007	successful genotyping(94%)	under follow-up care in2007, consent to participate (14%)
Adjustments, treatments	all mastectomy, randomized to chemotherapy and radiation therapy	adjusted for adjuvant therapy	none	none	restricted to guideline primary therapy, no chemotherapy	none	restricted to no chemotherapy in tamoxifen-treated	none	completed primary therapy, restricted to no chemotherapy	restricted to no chemotherapy
Adjustments, CYP2D6 inhibitors	none	excluded patients taking paroxetine	none	none	included in exposure definition	included in exposure definition	none	none	none	restricted to no SSRI
Adjustments, tamoxifen adherence	none	none	none	adjusted for tamoxifen duration	none	none	none	none	none	restricted to completed 5 years
Adjustments, prognostic markers	age, tumor size, node status	tumor size, node status, histologic grade, PR status, HER2 status	age, stage, race, ER status, PR status	tumor size, node status	tumor size, node status	node status	tumor size, node status	none	age, tumor size, node status, adjuvant therapy, surgery, C-erb2 status, ER status, PR status	age, menopausal status, tumor size, node status, nuclear grade, ER status, PR status, HER2 status

Abbreviations: ER = estrogen receptor; PR = progesterone receptor; SSRI = selective serotonin reuptake inhibitor; HER2 = human epidermal growth factor receptor 2