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CYP2D6 Genotype and Tamoxifen: Considerations for Proper Nonprospective Studies

MP Goetz[1,2] and **JN Ingle**[1]

[1]Department of Oncology, Mayo Clinic, Rochester, Minnesota, USA

[2]Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, Minnesota, USA

The International Tamoxifen Pharmacogenomics Consortium (ITPC) was formed to assess the relationship between *CYP2D6* genotype and tamoxifen therapy outcomes because of discordant findings in the published literature. The ITPC analyses provided clear insights into the importance of quality control in considering the essential factors that are necessary to answer this pharmacogenetic question and, by extension, the precautions that must be considered for proper retrospective and "prospective– retrospective" studies.

There has been great controversy surrounding the role of *CYP2D6* variation and tamoxifen efficacy. Following initial data demonstrating an association between *CYP2D6* genotype and clinical outcomes,[1,2] a 2006 special-emphasis panel from the US Food and Drug Administration recommended changing the tamoxifen label to incorporate data that *CYP2D6* genotype was an important biomarker associated with tamoxifen efficacy. Since then, multiple conflicting studies have delayed the label change, and results from secondary analyses of prospective clinical trials evaluating five years of tamoxifen administration were expected to clarify the controversy. The Breast International Group 1–98 (BIG 1–98) trial[3] and the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial[4] simultaneously published negative results in 2012, demonstrating no evidence for an association between *CYP2D6* genotype and recurrence. However, letters to the editor identified critical design flaws in both studies, including the observation of massive deviation from Hardy-Weinberg equilibrium (HWE) ($P = 2.5 \times 10^{-92}$) in the BIG 1–98 trial and the fact that in the ATAC trial less than 19% ($n = 588$) of the patients randomized to tamoxifen were analyzed. The reasons for the deviation in HWE were attributed in part to the use of nonstandard PCR techniques and the use of somatic DNA derived from breast tumor cores (instead of germline DNA), contraindicated given the frequent loss of heterozygosity known to occur at the *CYP2D6* locus.[5–7]

By contrast, a secondary analysis of the Austrian Breast and Colorectal Cancer Study Group (ABCSG 8) trial, which compared 5 years of tamoxifen with sequential tamoxifen followed

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Correspondence: MP Goetz (goetz.matthew@mayo.edu).

CONFLICT OF INTEREST

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by anastrozole, demonstrated that, compared with CYP2D6 extensive metabolizers (EMs), *CYP2D6* poor metabolizers (PMs) had a significantly higher rate of recurrence and death—but only in patients treated with tamoxifen mono-therapy and not anastrozole, a drug not metabolized by CYP2D6 (ref. 8). Notably, *CYP2D6* genotype was within HWE in this study.

Published data from the ITPC[9] provided additional insight into this controversial area. The ITPC was formed with the intent to aggregate, curate, and analyze the CYP2D6 data available from published breast cancer studies to answer the following question: should *CYP2D6* genotyping guide the use of tamoxifen in breast cancer? The ITPC demonstrated that in postmenopausal women with estrogen receptor (ER)- positive breast cancer receiving 20 mg/ day tamoxifen for 5 years (criterion 1, n = 1,996), CYP2D6 PM status was associated with worse invasive disease–free survival (hazard ratio (HR) = 1.25; 95% confidence interval (CI) 1.06–1.47; P = 0.009). However, *CYP2D6* genotype was no longer statistically significant when tamoxifen duration, menopausal status, and annual follow-up were not specified (criterion 2, n = 2,443; 49%; HR 1.17, 95% CI 0.90–1.52, P = 0.25) nor when no exclusions were applied (criterion 3, n = 4,935; 99%; HR 1.07; CI 0.92–1.26; P = 0.38). These three criteria were developed to allow analysis of a maximum number of samples but required a progressive loosening of requirements going from criterion 1, which approximates the eligibility requirements intended when the ITPC was originally formed (Supplementary Information online), to criterion 3, which includes all ITPC data.

There are two ways to interpret the ITPC data. One is that because criteria 2 and 3 demonstrated no association between *CYP2D6* genotype and invasive disease–free survival, the results are “null” and these results “validate” other negative studies. However, ITPC investigators demonstrated that a test of “homogeneity of the estimates” across sites suggested that the meta-estimate and its association P value were suspect for criteria 2 and 3, and therefore urged caution before making conclusions from criteria 2 and 3. Another interpretation is that criterion 1 results provide further evidence in support of *CYP2D6* as an important biomarker. Although there was no indication for heterogeneity for criterion 1 (P = 0.899), and criterion 1 inclusion criteria were identical to those of the CYP2D6 analysis of the ABCSG 8 trial,[8] there has been criticism from Dr. Berry[10] that criterion 1 was developed “*ad hoc*,” following an initial negative presentation of uncurated data from the entire ITPC cohort at the 2009 San Antonio Breast Cancer Symposium.[11] However, it is important to note that an analysis plan was developed before Dr. Berry’s involvement that, in fact, is the essence of criterion 1 (Supplementary Information online).

A critical analysis of the CYP2D6 tamoxifen literature raises the following question: what study designs should be considered in the “prospective- retrospective” studies in which the original study was not designed to answer the pharmacogenetic question? If CYP2D6 is simply the measurement of a tumor prognostic biomarker, one could approach the CYP2D6 question as for any other tumor biomarker, with the starting point being access to tumor DNA from data sets containing tamoxifen-treated patients. However, if the scientific question relates to whether exposure to endoxifen (the major active metabolite of

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tamoxifen), approximated by *CYP2D6* genotype, is associated with tamoxifen efficacy, then basic pharmacology principles must guide study design.

So what are these basic pharmacology principles? To review, the area under the curve is the integral of the concentration– time curve, which is a function of the concentration of the drug over time. There are a multitude of genetic and environmental factors that alter the area under the curve related to absorption, distribution, metabolism, and excretion. Whereas the tamoxifen *CYP2D6* literature has focused on the genetic factors that alter hepatic metabolism and thus endoxifen exposure, there is great heterogeneity in the tamoxifen pharmacology literature (and the data supplied to the ITPC) regarding additional critical pharmacological factors that alter endoxifen exposure, including (i) tamoxifen dose, (ii) duration of tamoxifen treatment, and (iii) use of drugs other than tamoxifen (e.g., aromatase inhibitors, CYP2D6 inhibitors). Let's put these principles into the context of tamoxifen and its metabolism.

Tamoxifen is a weak antiestrogen; however, both 4-hydroxy metabolites result in 100-fold greater inhibition of estrogen-mediated stimulation of breast cancer cells compared with tamoxifen.[12] Because 4-hydroxy tamoxifen concentrations are low (3–5 nmol/l), with little variability, attention has shifted to whether variation in endoxifen steady-state concentrations (C_{ss}) are associated with tamoxifen efficacy. Following initial observations that *CYP2D6* genotype and CYP2D6 inhibitors were associated with low endoxifen C_{ss} (ref. 13), prospective studies using the same dose (20 mg/day) have validated these findings, demonstrating that endoxifen C_{ss} ranges from 5 to 10 nmol/l in *CYP2D6* PMs to as high as 80 nmol/l in CYP2D6 EMs or ultrarapid metabolizers not receiving a CYP2D6 inhibitor. Preclinical models demonstrated that variation within the range of human endoxifen plasma concentrations (5–80 nmol/l) led to important effects on estrogen-mediated breast cancer proliferation. Specifically, endoxifen concentrations of 5–10 nmol/l were ineffective in tumor cells exposed simultaneously to tamoxifen and its metabolites; by contrast, a stepwise increase in endoxifen concentrations resulted in significantly greater reductions in estrogen-mediated proliferation.[12]

Considering the first element, dose: is there evidence that increasing the tamoxifen dose increases endoxifen C_{ss} into a range associated with greater antitumor activity? The answer is provided from a prospective study by Irvin *et al*., in which an increase in dose from 20 to 40 mg/day (another US Food and Drug Administration–approved dose) increased endoxifen C_{ss} in CYP2D6 PMs but not in CYP2D6 EMs.[14] It follows that a major design flaw in studying a pharmacology biomarker would be to not control for dose.

What about duration and the use of aromatase inhibitors after tamoxifen? There are extensive data showing that the benefits of tamoxifen, when dosed in the adjuvant setting, increase in proportion to duration of administration, with duration of 10 years > 5 years > 3 years > 2 years > 1 year > no treatment. A secondary analysis of the ABCSG 8 trial demonstrated no association between *CYP2D6* genotype and recurrence in the setting of a short duration of tamoxifen followed by anastrozole but a significant association in the setting of the five-year duration.[8] This latter observation may relate as much to an expected low event rate during the first two years as it does to relative benefit of tamoxifen. However,

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a close evaluation of this trial demonstrated that the detrimental effect of CYP2D6 poor metabolism was maintained throughout the duration of tamoxifen therapy but was lost when anastrozole was administered after tamoxifen. It follows that, in retrospective study designs, it would be critical to control for the administration of an active drug such as an aromatase inhibitor, which alters the hazard for an event when administered after tamoxifen.

Another critical factor that determines endoxifen metabolite exposure is drug-induced inhibition of the CYP2D6 enzyme.[13] The relative importance of CYP2D6 inhibitors is related to the duration of overlap with tamoxifen. This was demonstrated by Kelly *et al.*, who found that longer (but not shorter) duration of overlap between the inhibitor and tamoxifen use was associated with an increased risk of breast cancer deaths.[15] Within the ITPC, information regarding coadministration of drugs was not available for most patients.

A note should be made about genotyping fidelity and the ability to accurately predict endoxifen exposure. Many early studies assessed a limited number of *CYP2D6* alleles, leading to a misclassification of the CYP2D6 and thus endoxifen- exposure phenotypes. This leads to a particular type of bias in which undetected PMs are falsely assigned to the EM or IM groups. Additionally, as outlined above, most studies collected neither plasma samples (to assess endoxifen concentrations) nor lymphocytes (to extract germline DNA). A great deal of literature has already been published regarding the importance of using germline DNA to avoid genotyping errors.[7] However, it must be emphasized that plasma endoxifen exposure is approximated by germline (not somatic) *CYP2D6* genotype. Therefore, studies that demonstrated substantial evidence for genotyping error as assessed by HWE cannot be used to either support or refute the CYP2D6 hypothesis.

Finally, equally important to controlling for factors that alter endoxifen C_{ss} is controlling for other cancer therapies known to alter the hazard for recurrence. Because the underlying biological hypothesis is that *CYP2D6* PMs have a higher risk of recurrence because the tumor is being exposed to a weak anti-estrogen (tamoxifen) without endoxifen, the effects of lower endoxifen C_{ss} may be irrelevant in the setting where additional active therapies (chemotherapy, anti- HER2 drugs, or aromatase inhibitors) are administered either before, during, or after tamoxifen.[8] Some of the pharmacological, epidemiological, and genotyping issues observed in the CYP2D6 literature are summarized in Table 1.

In summary, the analyses of the ITPC are particularly informative with regard to the potential limitations of retrospective and “prospective–retrospective studies” that to-date have formed the basis for assessing the relationship between *CYP2D6* genotype and outcomes with tamoxifen therapy. That is, if attention isn’t paid to the essential factors related to the pharmacogenetic question, the analysis will be irretrievably flawed. In the case of a drug exposure question, similar doses, duration, and control for other factors that affect drug exposure must be considered, while still controlling for the factors that affect tamoxifen end points such as ascertainment of the drug target (ER) (tamoxifen is ineffective in an ER-negative setting) and mandating standards for follow-up of patients. Whereas most consider these irrefutable requirements, only 1,996 (40%) of the 4,935 ITPC patients fulfilled these most basic requirements, suggesting that some reviewers and editors do not understand the elements required to answer a pharmacogenetic drug exposure question.

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Therefore, given that a secondary analysis of the ABCSG 8 clinical trial fully validated that *CYP2D6* genotype is associated with an increased rate of recurrence or death, we recommend that women who meet the criteria as outlined in the ABCSG 8 trial (identical to criterion 1 in the ITPC study[9]) be counseled regarding the potential impact of *CYP2D6* genotype on the effectiveness of adjuvant tamoxifen, and potent CYP2D6 inhibitors should be avoided in these patients.

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Table 1

A summary of some of the factors associated with variability in the published CYP2D6 tamoxifen studies

Factor	Effect
Pharmacology factors	
Variability in rates of adherence among different *CYP2D6* genotypes	Higher rates of nonadherence in CYP2D6 EMs given the greater likelihood of side effects
Duration of tamoxifen administration	Duration must be consistent because tamoxifen is more effective with longer duration than shorter duration, and different durations could obscure any impact of CYP2D6
Dose (40 vs. 20 mg/day)	Increase in endoxifen C_{ss} in CYP2D6 PMs (but not EMs) with higher doses of tamoxifen, thereby decreasing the risk associated with the PM state[14]
Concurrent administration of potent CYP2D6 inhibitors	CYP2D6 EMs converted to PMs in the presence of CYP2D6 potent inhibitors
Clinical factors	
Chemotherapy administered before or after tamoxifen	Fewer recurrences and fewer at-risk patients; loss of statistical power
Administration of aromatase inhibitor after tamoxifen	CYP2D6 is not responsible for the metabolism of aromatase inhibitors, and the effect of CYP2D6 metabolism is lost in patients switched to an aromatase inhibitor.[8], Lack of knowledge regarding switching to an aromatase inhibitor can obscure CYP2D6 effect
Inclusion of ER-negative patients	Tamoxifen does not reduce the risk of recurrence of ER-negative breast cancer
Genotyping factors	
Use of tumor cores for determination of *CYP2D6* genotype	*CYP2D6* genotype from tumor-derived DNA subject to error due to somatic loss of heterozygosity affecting the 22q13 *CYP2D6* locus[3,5,6,7]
Limited *CYP2D6* allele coverage (genotyping for only the *4 allele)	Misclassification of CYP2D6 PMs, thereby falsely assigning undetected PMs to the EM or IM groups[5]
Use of nonstandard genotyping techniques (60 PCR cycles)	Potential for genotyping error[3]

C_{SS}, steady-state concentration; EM, extensive metabolizer; ER, estrogen receptor; IM, intermediate metabolizer; PM, poor metabolizer.