POINT/COUNTERPOINT

CYP2D6 Genotype Should Not Be Used to Determine Endocrine Therapy in Postmenopausal Breast Cancer Patients

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The antiestrogen tamoxifen is an effective treatment for all stages of estrogen receptor (ER)-positive breast cancer.[1] Tamoxifen blocks estrogen-dependent breast cancer growth by competing with estrogen for binding to its receptor. Tamoxifen itself has antiestrogenic properties, but it is metabolized by a number of cytochrome P450 enzymes into many different metabolites that have varying degrees of antiestrogenic activity. Studies have consistently shown that cytochrome P450 2D6 (CYP2D6), a highly polymorphic drug-metabolizing enzyme, is the enzyme primarily responsible for the production of one of the most potent metabolites, endoxifen (4-hydroxy-*N*-desmethyl-tamoxifen). Preclinical studies of estrogen-dependent breast cancer have shown that endoxifen is an approximately 100-fold more potent antiestrogen than tamoxifen or the major tamoxifen metabolite, *N*-desmethyl-tamoxifen. Patients who are homozygous for two null *CYP2D6* alleles, and are therefore poor metabolizers (PMs), as well as patients who are taking medications that are CYP2D6 inhibitors, have lower serum endoxifen concentrations as compared with patients who have one or more wild-type *CYP2D6* alleles (intermediate metabolizers (IMs) and extensive metabolizers (EMs), respectively) (reviewed by Hertz *et al.*[2]).

These data led to the hypothesis that CYP2D6 PMs may not respond to tamoxifen therapy and that they may experience fewer side effects, compared with EMs, because they have lower circulating endoxifen levels. In an initial study testing this hypothesis, the relapse-free time and disease-free survival were lower in CYP2D6 PMs than in EMs.[3] This report spurred subsequent, highly heterogeneous studies of patients treated with tamoxifen. To date, the studies have produced very contradictory results, with some studies showing the hypothesized positive association between *CYP2D6* genotype and tamoxifen response, others showing no association, and some even suggesting an inverse association.[2] Most of these reports come from studies of convenience, representing quite low levels of evidence, and only a few of the studies utilized specimens collected and archived from prospectively conducted clinical trials, the gold standard for studies with a high level of evidence.

Despite this uncertainty, Ratain (as in his Counterpoint)[4] and others argue that there is sufficient evidence to change clinical practice to include *CYP2D6* genotype in order to guide decisions regarding tamoxifen treatment in postmenopausal women with ER-positive breast cancer. Evidence in support of using *CYP2D6* genotype in this way comes largely from the study published by Schroth *et al.*, which showed that PM patients had shorter event-free and disease-free survival, but not shorter overall survival, compared with IMs and EMs.[5] This cohort, which did not contain a control group, represents a mixed data set of previously reported and new additional cases, using data sets of convenience and DNA derived from formalin-fixed, paraffin-embedded (FFPE) tumor, blood, and fresh-frozen tumor. Evidence against changing clinical practice to include *CYP2D6* testing has been generated by a number of studies, including two prospective–retrospective studies—by the Breast International Group (BIG) 1-98 and the Arimidex, Tamoxifen, Alone or in Combination (ATAC) clinical trialist—that failed to show an association between *CYP2D6* genotype and clinical outcomes in tamoxifen-treated patients.[6,7] The strengths of these two studies include the large number of patients analyzed, the long-term and detailed clinical follow-up data within registration clinical trials, and the inclusion of control groups (patients receiving aromatase inhibitors, AIs). To date, the available clinical data do not achieve the level of evidence required to change clinical practice to include *CYP2D6* genetic testing for women with ER-positive breast cancer considering adjuvant tamoxifen therapy.

Proponents of the CYP2D6–tamoxifen hypothesis have noted that the *CYP2D6* genotype data from BIG1-98 and ATAC are out of Hardy-Weinberg equilibrium (HWE) and raised concerns about the use

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of archival tumor samples as the source of DNA. Although HWE testing is a common practice for assessing systematic genotyping errors, straightforward HWE testing for CYP2D6 is complicated by germline copy-number variations observed in *CYP2D6* (mainly *CYP2D6*5* gene deletion and *CYP2D6*2* gene amplifications) and by population admixture. Indeed, even if one considers HWE an indication of adequate *CYP2D6* genotype distribution, then the issue of deviation from HWE is common to many CYP2D6 tamoxifen studies. For example, in the study by Goetz *et al.*,[3] although not reported, one can calculate a significant deviation from HWE with *CYP2D6*4* ($\chi^2 = 13.5$, $P = 0.00024$). An early report by Schroth *et al.*, representing a German data set not taken from a prospective trial, states that "Two polymorphisms, *CYP2D6*10* and *CYP2C9*2*, were not in Hardy-Weinberg equilibrium"; however, the specific *CYP2D6* genotype data are not provided, prohibiting independent analyses of HWE.[8] In what purports to be a larger validation study,[5] the cohorts previously reported by Goetz *et al.*[3] and Schroth *et al.*[8] were combined with additional samples from a hospital registry. Not unexpectedly, statistically significant departures from HWE in *CYP2D6*4* ($\chi^2 = 6.9$, $P = 0.00855$) and *CYP2D6*10* ($\chi^2 = 27.4$, $P = 1.65 \times 10^{-7}$) were observed.[5] Thus, lack of HWE is present both in studies supporting an association between CYP2D6 and tamoxifen and in those not supporting such an association.

In all pharmacogenetic studies, it is of primary importance that the genotype data be as accurate as possible, and in the case of *CYP2D6*, they should accurately represent a patient's CYP2D6 metabolic phenotype. CYP2D6 is one of the most widely studied P450 enzymes, and the distribution of *CYP2D6* genotype and phenotypes across multiple different ethnic groups has been established. An examination of the *CYP2D6* genotype frequencies and the distribution of their corresponding CYP2D6 metabolic phenotypes in the major CYP2D6–tamoxifen studies demonstrates that all fall within the expected range (**Table 1**). Earlier studies tested only a few *CYP2D6* alleles, which nevertheless captured the majority of PM patients. In later studies, with more *CYP2D6* alleles tested, a small number of additional PMs would be expected along with a better delineation between IMs and EMs. All studies have the expected *CYP2D6* genotype and phenotype distributions based on the alleles that were assayed. Therefore, it is inconsistent to argue that HWE deviations invalidate the BIG1-98 and ATAC studies, but not the studies by Goetz *et al.* and Schroth *et al.*, because they all apparently deviate from HWE. Given that all studies have the expected number of patients who are CYP2D6 PMs, IMs, and EMs, it is reasonable to assume that they present valid *CYP2D6* genotype data and that the differences in the associations between CYP2D6 and tamoxifen response are due to factors other than genotype errors. These possible factors include differences in patient selection, disease stage, intrinsic subtype, additional treatments and medications, and the quality of the follow-up data.

Nonetheless, Ratain and colleagues have suggested that genotypes obtained from FFPE tumor tissue do not faithfully represent the germline DNA, in that somatic deletions due to loss of heterozygosity (LOH) of the *CYP2D6* chromosomal locus (22q13) are well established in breast cancer.[9] Earlier studies have provided evidence for deletions of 22q13 in primary breast cancers,[10] but they used low-resolution methodologies that fail to detect the specific genes within the large 22q13 region that exhibit LOH. More recent studies conducting very-high-resolution fine genomic mapping to analyze much larger numbers of primary breast tumors have failed to show frequent LOH of the *CYP2D6* locus in the majority of breast cancer tumor types. One study suggested occasional LOH in the *CYP2D6* gene region, but this observation was confined to basal-like breast cancers, which are rarely ER-positive, and is therefore irrelevant in this debate.[11]

Finally, even if LOH in *CYP2D6* were a frequent occurrence in ER-positive breast tumors that are available for genotyping, tumors always contain normal tissue that provides DNA containing two copies of each allele. Taken together, the data argue against Ratain's concern that genotyping errors occurred in the studies that used FFPE tumor tissue for genotyping. His assertion that tumor LOH confounded germline *CYP2D6* gene testing in the BIG1-98 study is a hypothesis that is worthy of testing, but, unless it is proven, the hypothesis itself does not invalidate the data presented.

Other pharmacological concerns must also be considered in this debate. Tamoxifen is not a true "prodrug," because the parent drug and the many other metabolites that are produced independent of CYP2D6 have antiestrogenic activity. Furthermore, even PM patients make some endoxifen that, combined with tamoxifen and its other metabolites, might be present at sufficient concentrations to block ER signaling. Despite the inconsistent clinical data and these biological and pharmacokinetic considerations, Ratain and his colleagues maintain that all ER-positive patients should undergo *CYP2D6* genotype

Table 1 *CYP2D6* genotype frequencies and distribution of corresponding CYP2D6 metabolic phenotypes in the major CYP2D6–tamoxifen studies

	CYP2D6 phenotype			
Study	**PM**	**IM**	**EM**	***CYP2D6* alleles tested**
Goetz *et al.*[3]	6.8	21.1	72.1	*4, *6
Schroth *et al.*[8]	7.1	33.0	59.9	*4, *5, *10, *41
Schroth *et al.*[5]	6.0	48.1	46.0	*3, *4, *5, *10, *41, XN
Rae *et al.*[7]	6.5	39.6	53.9	*2, *3, *4, *6, *10, *41
Regan *et al.*[6]	8.3	29.5	62.2	*3, *4, *6, *7, *17,

EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer.

to guide adjuvant endocrine therapy. Given that several studies (including ATAC and BIG1-98) have demonstrated that the AIs are slightly more effective than tamoxifen,[1] one could argue that we should abandon tamoxifen altogether and that all postmenopausal women should receive AI therapy. However, these arguments ignore the extensive use of tamoxifen in premenopausal women (for whom AIs are contraindicated) and for postmenopausal women who cannot tolerate an AI (which may exceed 30% of those treated). A decade ago, we hypothesized that *CYP2D6* genotype could help guide antiestrogen therapy decisions based on sound pharmacokinetic data. However, in retrospect, this hypothesis appears to be based on a weak biological underpinning, and the available clinical data are insufficient to support it.

In conclusion, to date, the weight of all available evidence argues against the use of *CYP2D6* genotype testing for women considering tamoxifen. Although higher levels of evidence addressing this issue are desirable, until they become available *CYP2D6* genetic testing should not be introduced into routine clinical practice to guide the decision as to whether a patient should receive tamoxifen therapy.

CONFLICT OF INTEREST

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CYP2D6 Genotype and Tamoxifen Activity: Understanding Interstudy Variability in Methodological Quality

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There has been great controversy over the years regarding the impact of *CYP2D6* polymorphisms on the efficacy of tamoxifen in women with breast cancer. The most significant publication to date is the report by investigators in Stuttgart, Germany, and at the Mayo Clinic of a 1,325-patient study, published in 2009 in the *Journal of the American Medical Association*.[1] Despite this high-impact publication, there has been variable acceptance of these findings because of inconsistent replication of the results.[2]

The controversy

Are the studies demonstrating an association between *CYP2D6* genotype and tamoxifen efficacy false-positive studies? We acknowledge that false-positive studies are endemic in the pharmacogenomic literature, generally because of failure to correct for multiple testing of associations between a large number of candidate polymorphisms and multiple phenotypes. However, this concern is not applicable to the study by Schroth and colleagues[1] or most of the other positive studies that have focused exclusively on testing of a single polymorphic gene, *CYP2D6*. Furthermore, although some of these positive studies have modest sample sizes and/or incomplete genotyping, those factors would not affect the probability that a positive study is a true positive (but would increase the probability of a false-negative study).

Or are the studies that failed to demonstrate such an association false-negative studies? If so, what would be the explanation? False-negative replication studies can occur for many reasons. One often focuses on statistical explanations for failure of replication. For example,

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