The role of common and rare genetic variation in CYP2D6 in determining response to tamoxifen therapy in women with breast cancer has been controversial for nearly a decade. In two recent articles by Regan et al. (1) and Rae et al. (2), no association between CYP2D6 metabolizer status predicted on the basis of genotype and outcome in women with breast cancer treated with adjuvant hormone therapy was demonstrated. In an accompanying editorial (3), Kelly and Pritchard suggest that the controversy has finally been laid to rest.

So what is different about the null findings of the new studies (1,2) that ends a controversy that previous studies have not [eg, (4,5)]? A key factor influencing the judgment of Kelly and Pritchard seems to be the fact that the studies were nested within randomized controlled trials. Indeed, they state that an important lesson to be learned from the controversy is that the use of randomized trials prospectively designed and retrospectively analyzed is critical to assess the role of biomarkers such as CYP2D6. The assumption that randomized trials are required to avoid bias in this context is a fundamental error in understanding of basic epidemiological principles. The primary purpose of randomization is to minimize the chance of correlation between the exposure of interest and potential confounding variables by randomly assigning the exposure of interest. In a genetic association study, the exposure of interest is the genotype, not the therapeutic arm. It is highly unlikely, but possible, that genotype will be correlated with other possible confounding variables. The two new studies are not randomized controlled trials of the exposure of interest. Indeed, it is not possible to randomize germline genotype or any other biomarker. One potential benefit of evaluating the role of germline genotype or other biomarker for outcomes by nesting the study within a randomized controlled trial of another exposure is that other important factors may have been carefully measured. We believe that purely observational studies may have equally good standardization of the population of interest and the outcome of interest.

Kelly and Pritchard did not discuss some flaws in the study designs that may render the findings invalid. A major limitation of both new studies is the use of tumor DNA to determine germline genotype. One measure of genotyping quality is duplicate concordance. This was not reported by Regan et al. (1). Consistency of genotype frequencies with those expected under Hardy–Weinberg equilibrium (HWE) is another measure of genotyping quality that ought to be standard reporting practice. The proportions of the three CYP2D6*4 genotypes (rare homozygous [8.6%], heterozygous [20.5%], and common homozygous [70.9%]) in 3828 patients are provided in Table 2 (1). A standard chi-squared test shows highly statistically significant deviation of these frequencies from those expected under HWE (χ² = 416, P = 10−92). Deviation of this magnitude would suggest a severe measurement error. There are two possible reasons for this problem. First, CYP2D6 has a pseudogene, and it is known that this pseudogene can interfere with an assay for the CYP2D6*4 allele. Second, loss of heterozygosity in a tumor would result fewer heterozygotes than expected, as is observed here. Rae et al. (2) mentioned that the allele frequencies were in HWE. This statement is technically incorrect as HWE relates to genotype frequencies and not allele frequencies. Furthermore, the test statistic and P value are not given and genotype frequencies are not provided, so it is not possible for the reader to verify the calculations. The Rae et al. study (2) comprised 588 patients treated with tamoxifen, of whom just 70 died from breast cancer during follow-up.

No power calculation was given, but a study of this size would have approximately 50% power at a type I error rate of 0.05 to detect a hazard ratio of 1.5 in a high-risk group comprising 30% of the cohort.

We published a large observational study that comprehensively evaluated CYP2D6 genotypes in a large, well-conducted, but not perfect, observational study 2 years ago (4). In terms of patient sample numbers (3155 patients), our study was larger than any previously published and is larger than both the new studies combined. We also found no evidence for predicted CYP2D6 metabolizer status and breast cancer–specific mortality. The article was not cited in either of the new articles or the editorial. Further support for our findings was published by Lash et al., although this study also suffered from the limitation of using tumor-derived DNA (5). If the valid results from observational studies had ended the controversy, some patients may have been spared the unnecessary expense of having a test of no clinical utility.

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References

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in tumors from which DNA samples were obtained may account for these flawed results. The estimated excess of homozygotes is approximately 5% for each genotype, consistent with approximately 33% of tumor samples having CYP2D6 deletions. Because CYP2D6 is located on chromosome 22q13 where frequent losses of heterozygosity in breast cancer cells have been reported (6), it would not be surprising if CYP2D6 were deleted in breast cancer. In addition, 22q13 deletions have been associated with a worse prognosis, as exemplified by a large single-institution Japanese study in which 32% of tumors had 22q13 deletions (7). Thus, if a tumor from a patient who is a germ-line heterozygote loses one of the alleles, this causes misclassification of that patient’s tamoxifen metabolism phenotype. An alternative explanation, given the incomplete genotyping in these DNA samples, is that samples from heterozygotes are disproportionately not called (ie, the missing data are not missing at random). Genotyping of additional markers on chromosome 22q13 could distinguish these hypotheses. In any case, the genotype data from this study fail the most rudimentary quality tests, and therefore, we question its validity. Given the importance of the question being studied, we urge the retraction of the Regan et al study (1).

We also urge reanalysis of other studies that have utilized tumor DNA for genotyping, given the potential for hemizygous deletion of CYP2D6 in breast cancer. Hopefully, this will be another important “lesson learned” for investigators in breast cancer genomics (3). The goal of personalized medicine is to provide an appropriate dose of the optimal drug to each individual patient, but it is critical that quality data from rigorous studies be used to inform these decisions.

Notes
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Re: CYP2D6 Genotype and Tamoxifen Response in Postmenopausal Women With Endocrine-Responsive Breast Cancer: The Breast International Group 1-98 Trial
Two recent articles by Regan et al. (1) and Rae et al. (2), accompanied by an editorial (3), purport to settle the controversy of whether CYP2D6 genotype is associated with the pharmacodynamics of tamoxifen. There have been many previous studies, which vary in source of DNA (tumor, blood), phenotype (efficacy, toxicity, pharmacokinetics), general design (prospective, retrospective), concomitant medications (other anticancer medications, CYP2D6 inhibitors), and statistical approaches (4). A key issue in all genetic studies is the quality of the primary genetic data, as no inferences can be drawn from genotype data of low quality. In regard to the latter, a critical and fundamental first step in assessing the quality of genotypes is a test for deviation of the genotype distribution from Hardy–Weinberg equilibrium (HWE) (5), which should be considered of particular importance when DNA for genotyping has been extracted from tumor, rather than germ-line tissue.

Thus, it is of grave concern that one of the recent studies (1) shows clear evidence of massive departures from HWE; insufficient information was provided in the second study (2) to assess the quality of the genotype data. Using the data in Table 2 of the Regan et al study (1), the two most important variants, rs3892097 and rs28371725, fail quality control, with unacceptable P values (from χ² tests for consistency with HWE) of approximately 10⁻¹⁰ and 10⁻¹⁷, respectively.

For both variants, there is an excess of homozygotes, consistent with the hypothesis that hemizygous deletions of CYP2D6 in tumors from which DNA samples were obtained may account for these flawed results. The estimated excess of homozygotes is approximately 5% for each genotype, consistent with approximately 33% of tumor samples having CYP2D6 deletions. Because CYP2D6 is located on chromosome 22q13 where frequent losses of heterozygosity in breast cancer cells have been reported (6), it would not be surprising if CYP2D6 were deleted in breast cancer. In addition, 22q13 deletions have been associated with a worse prognosis, as exemplified by a large single-institution Japanese study in which 32% of tumors had 22q13 deletions (7). Thus, if a tumor from a patient who is a germ-line heterozygote loses one of the alleles, this causes misclassification of that patient’s tamoxifen metabolism phenotype. An alternative explanation, given the incomplete genotyping in these DNA samples, is that samples from heterozygotes are disproportionately not called (ie, the missing data are not missing at random). Genotyping of additional markers on chromosome 22q13 could distinguish these hypotheses. In any case, the genotype data from this study fail the most rudimentary quality tests, and therefore, we question its validity. Given the importance of the question being studied, we urge the retraction of the Regan et al study (1).

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References

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