Re: CYP2D6 Genotype and Tamoxifen Response in Postmenopausal Women With Endocrine-Responsive Breast Cancer: The Breast International Group 1–98 Trial

A recent article by Regan et al. (1) on tamoxifen pharmacogenetics, although conducted at a high standard in most respects, presents highly implausible CYP2D6 genotyping results that raise serious doubt about the conclusions. Determining constitutional genotypes from formalin-fixed paraffin-embedded (FFPE) tumor tissue is technically challenging, particularly with a complex gene like CYP2D6 that exhibits copy number variation and is flanked by two highly similar pseudogenes. The potential for genotyping errors attributable to allele loss in breast cancer must also be considered. One widely recognized method for quality control of genotyping data is to determine whether allele frequencies conform to Hardy–Weinberg proportions. Hence the genotype frequencies reported by Regan et al. were tested for Hardy–Weinberg equilibrium (HWE) using the Pearson χ² test (2). The results are presented in Table 1. The probability of the reported genotype frequencies is astronomically small (e.g., the probability of the reported CYP2D6*4 allele proportions is 2.5 × 10⁻³⁰). What might account for these results? One clue is the consistent excess of homozygotes (especially for the minor allele) and deficiency of heterozygotes, compared to what would be expected if the alleles were in HWE. The depletion of heterozygotes is likely attributable, at least in part, to the use of tumor DNA. The area representative of the invasive tumor component should have been avoided in Regan et al. (1) because loss of heterozygosity (LOH) involving chromosome 22 generally, and band 22q13.1 in particular (the location of CYP2D6), has been widely reported in breast cancer. One detailed study of chromosome 22 alterations in breast cancer reported monosomy in 7 of 63 patients (11%) and other genomic imbalances affecting 22q in a further 6 patients (overall 13 of 60 patients had 22q genomic imbalances) (3). Although it is true that several studies have shown high concordance of genotypes from normal DNA and FFPE-derived tumor DNA, including CYP2D6 (4, 5), those studies have generally used whole FFPE sections for DNA isolation. Because of the infiltrative nature of breast cancer, a typical paraffin block will almost invariably contain a substantial proportion of stromal and normal cells. However, in coring out a 1-mm tumor-enriched region, Regan et al. (1) reduced the proportion of normal DNA, perhaps to undetectable levels.

Table 1. Test of genotype proportions reported in Regan et al. (1) for conformity to Hardy–Weinberg proportions

<table>
<thead>
<tr>
<th>Allele</th>
<th>Inferred genotype frequencies: (genotype proportions) × (No. of subjects)</th>
<th>Expected genotype frequencies given reported allele frequencies</th>
<th>Probability of reported genotype proportions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Homozygous</td>
<td>Heterozygous</td>
<td>Wild-type</td>
</tr>
<tr>
<td>CYP2D6*4</td>
<td>329.2</td>
<td>784.7</td>
<td>2714.1</td>
</tr>
<tr>
<td>CYP2D6*41</td>
<td>161.4</td>
<td>322.7</td>
<td>3357.9</td>
</tr>
<tr>
<td>CYP2D6*3</td>
<td>12.0</td>
<td>392.7</td>
<td>2942.7</td>
</tr>
<tr>
<td>CYP2D6*6</td>
<td>5.4</td>
<td>89.3</td>
<td>2612.3</td>
</tr>
<tr>
<td>CYP2D6*17</td>
<td>370.2</td>
<td>808.9</td>
<td>1105.9</td>
</tr>
</tbody>
</table>

* The genotype data in Table 2 of Regan et al. (1) were presented in terms of the percentage of tested subjects who were homozygous for the mutant allele, heterozygous, or homozygous for the wild-type allele. By multiplying those percentages by the number of subjects successfully genotyped (also from Table 2 of Regan et al.), one can calculate the approximate number of subjects with each genotype (“Inferred genotype frequencies” in the table above). Those frequencies were used to calculate allele frequencies, which were then used to calculate “Expected genotype frequencies” using the Hardy–Weinberg equation. Finally, the probability of the reported genotype proportions from Regan et al. (1) (converted into inferred genotype frequencies) was estimated using the χ² distribution as described previously by Rodríguez et al. (2). Calculations were done using an online calculator created by Rodríguez et al. (2).

† P-values were determined from the χ² distribution; they are one-sided (right-tail). Two sided P-values would be double the values shown, which does not affect any of the conclusions.

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References


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ical utility. We were disappointed that the high levels of evidence demonstrating clin-
keen proponents of personalized medicine.
valuable part of peer review. We too are with comprehensive reporting of results as
We welcome the discourse that comes in tamoxifen-treated breast cancer patients.
Unfortunately, Nakamura et al. mis-

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Response
Pharoah et al., Nakamura et al., and Stanton have questioned the validity of two recent articles published in the Journal (1,2). They specifically question the geno-
typing quality in the Breast International Group (BIG) 1-98 study (1). Nakamura et al., in fact, urge the retraction of the BIG 1-98 study and reanalysis of all studies utilizing tumor DNA for genotyping. We welcome the discourse that comes with comprehensive reporting of results as a valuable part of peer review. We too are keen proponents of personalized medicine. However, we believe that adopting a new test into standard clinical care requires high levels of evidence demonstrating clinical utility. We were disappointed that the results of these two investigations did not support the hypothesis that CYP2D6 genotype was associated with the outcome of postmenopausal women with early breast cancer treated with tamoxifen. We stand firmly behind the quality of methodology of the BIG 1-98 study, the contribution of our results to the body of literature, and the value that the two investigations (1,2) together bring to informing the care of patients with breast cancer.

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When using DNA extracted from formalin-fixed paraffin-embedded (FFPE) tumor samples, could hemizygous somatic deletions of CYP2D6 in the tumor cells affect CYP2D6 genotype, as suggested by...