

## BRIEF COMMUNICATION

# Concordance Between *CYP2D6* Genotypes Obtained From Tumor-Derived and Germline DNA

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**Formalin-fixed, paraffin-embedded tumors (FFPETs) are a valuable source of DNA for genotype association studies and are often the only germline DNA resource from cancer clinical trials. The anti-estrogen tamoxifen is metabolized into endoxifen by *CYP2D6*, leading to the hypothesis that patients with certain *CYP2D6* genotypes may not receive benefit because of their inability to activate the drug. Studies testing this hypothesis using FFPETs have provided conflicting results. It has been postulated that *CYP2D6* genotype determined using FFPET may not be accurate because of somatic tumor alterations. In this study, we determined the concordance between *CYP2D6* genotypes generated using 3 tissue sources (FFPETs; formalin-fixed, paraffin-embedded unaffected lymph nodes [FFPELNs]; and whole blood cells [WBCs]) from 122 breast cancer patients. Compared with WBCs, FFPET and FFPELN genotypes were highly concordant (>94%), as were the predicted *CYP2D6* metabolic phenotypes (>97%). We conclude that *CYP2D6* genotypes obtained from FFPETs accurately represent the patient's *CYP2D6* metabolic phenotype.**

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DNA from formalin-fixed, paraffin-embedded tumors (FFPETs) has been used for “prospective–retrospective” (1) studies testing for associations between gene variants with cancer outcomes. Several studies have used FFPETs for germline *CYP2D6* genotyping to test for associations with response to tamoxifen therapy (2–8). Tamoxifen is metabolized into a more potent anti-estrogenic metabolite (endoxifen) by *CYP2D6* (9). Patients receiving tamoxifen who are homozygous for *CYP2D6* null alleles and classified as poor metabolizers (PMs) have substantially lower serum endoxifen concentrations than patients with heterozygous null (intermediate metabolizers [IMs]) and homozygous wild-type (extensive metabolizers [EMs]) alleles (10). Although it has been hypothesized that *CYP2D6* PMs do not benefit from tamoxifen therapy, studies testing this hypothesis have provided conflicting results [reviewed in (11)].

Results from two phase III studies (ATAC and BIG1-98), in which FFPETs were used for genotyping, failed to detect an association between *CYP2D6* and tamoxifen efficacy in postmenopausal, early-stage, estrogen receptor–positive breast cancer patients (7,8). Subsequently, three letters in the *Journal of the National Cancer Institute* suggested the *CYP2D6* genotypes from the BIG1-98 study might not reflect patients' germline *CYP2D6* genotypes because of a possible misclassification effect of somatic tumor alterations (12–14). Nakamura et al. suggested many (33%) breast tumors exhibit loss of one *CYP2D6* allele because of loss of heterozygosity (LOH) within the chromosomal region containing *CYP2D6*, resulting in the excess of *CYP2D6* homozygote genotypes, and deviations from Hardy–Weinberg equilibrium in the BIG1-98 trial (14). Previously, we showed that *CYP2D6* genotypes in a small sample set (n = 10) were 100% concordant between

FFPETs and whole blood cells (WBCs) (15). This study used whole tissue sections from FFPETs, which contain both tumor and normal cells, with the latter providing germline DNA that has not undergone somatic alterations. In contrast, the BIG1-98 study used DNA extracted from 1-mm diameter cores taken from malignant areas within the FFPETs. Given the clinical significance of the BIG1-98 investigation and other studies that have used FFPETs for *CYP2D6* genotyping (2–8), we investigated the concordance of *CYP2D6* genotypes from FFPETs and germline DNA derived from WBCs.

To assess *CYP2D6* genotypes from different tissue sources, DNA from FFPETs, formalin-fixed, paraffin-embedded unaffected lymph nodes (FFPELNs), and WBCs were analyzed from 122 breast cancer patients who provided written informed consent (Supplementary Table 1, available online). Three 0.6-mm diameter cores were taken from areas of high tumor burden within the FFPETs as identified by hematoxylin and eosin staining of the donor block. Three cores were also obtained from FFPELNs. Phlebotomy yielded WBC specimens. DNA was extracted from the 122 matched FFPETs, FFPELNs, and WBCs and genotyped for six *CYP2D6* single nucleotide polymorphisms using the Taqman allelic discrimination assays (Applied Biosystems, Foster City, CA) as previously described (8). In combination, these single nucleotide polymorphisms determine *CYP2D6*\*1, \*2, \*3, \*4, \*6, \*10, and \*41 genotypes. In addition, *CYP2D6* copy number variants (CNVs) were determined using DNA obtained from WBCs, as described previously (16) (Supplementary Material and Methods, available online).

*CYP2D6* allelic variants and genotype frequencies and tests for Hardy–Weinberg equilibrium deviation from the 122 WBCs are given (Supplementary Table 2, available online). We were unable to genotype one FFPET and five FFPELNs because of low DNA quality/concentration and one FFPELN for unknown reasons. Using *CYP2D6* genotypes from WBCs as our reference, concordance among formalin-fixed, paraffin-embedded samples providing quality DNA for *CYP2D6* genotyping

was 94.2% (n = 114 of 121; 95% confidence interval [CI] = 88.4% to 97.6%) and 97.4% (n = 114 of 117; 95% CI = 92.7% to 99.5%) for FFPEs and FFPELNs, respectively. Based on *CYP2D6* genotypes, samples were assigned *CYP2D6* phenotype scores of 0 (PM); 0.5, 1.0, and 1.5 (IM); and 2 (EM) (8). The concordance among formalin-fixed, paraffin-embedded samples providing quality DNA for *CYP2D6* score determination was 98.3% (n = 119 of 121; 95% CI = 94.2% to 99.8%) and 97.4% (n = 114 of 117; 95% CI = 92.7% to 99.5%) for FFPEs and FFPELNs, respectively (Figure 1).

Germline *CYP2D6* CNVs, both deletions and amplifications, have been identified (17). Currently, it is not feasible to test *CYP2D6* CNVs from formalin-fixed, paraffin embedded samples because the quality and quantity of the extracted DNA is below that required for quantitative polymerase chain reaction or direct sequencing of known splice sites. We determined the potential impact of the missing *CYP2D6* CNV information from FFPEs on concordance between formalin-fixed, paraffin-embedded samples and WBCs with known CNVs. In the 122 WBC DNA samples, we observed nine (7.4%; 95% CI = 3.4% to 13.5%) deletions and six (4.9%; 95% CI = 1.8% to 10.4%) amplifications in *CYP2D6*. All deletions and amplifications were in samples having 100% concordant

genotypes between WBCs, FFPEs, and FFPELNs. Adjusting *CYP2D6* scores to account for deletions, eight patients scored as 2 (EM) became 1 (IM), and one scored as 1 (IM) became 0.5 (IM). The six gene amplifications had only minimal impact on *CYP2D6* scores (Supplementary Table 3, available online). The missing CNV information from FFPEs and FFPELNs had no impact on predicting *CYP2D6* PM phenotypes.

Our data show that *CYP2D6* genotypes obtained from FFPEs and FFPELNs are more than 94% concordant with WBC genotypes. Furthermore, predicted *CYP2D6* metabolic phenotypes based on genotypes obtained from FFPEs and FFPELNs were more than 97% concordant with WBCs. Our data are consistent with an earlier study showing strong agreement between *CYP2D6* genotypes using DNA extracted from archived breast tumors and normal lymph nodes (18). These data refute the concern that breast tumor LOH causes patients to be misclassified when using FFPEs for *CYP2D6* genotyping. Of note, we observed similar rates of discordance between FFPEs and FFPELNs versus WBCs. Given that these study lymph nodes contain solely normal tissue, we conclude that the minor genotype disagreements observed in our study are due to the technical challenges

of *CYP2D6* genotyping using DNA of low quality or quantity of DNA obtained from formalin-fixed, paraffin-embedded tissues or both.

Although earlier studies have suggested that the chromosomal region containing the *CYP2D6* gene (22q13) undergoes LOH in breast tumors, these studies analyzed only a small number of tumors and failed to conduct the genomic fine mapping required to identify the specific genes exhibiting LOH (19,20). Recently, large fine gene mapping studies generating very high resolution data failed to show substantial LOH in *CYP2D6* in the majority of breast cancer tumor types (21,22).

These data, along with those from the large breast cancer genomic studies that failed to detect LOH in the *CYP2D6* locus, demonstrate that *CYP2D6* genotypes obtained from FFPEs are suitable for *CYP2D6* germline genotype determinations. With less than 10% estimated misclassification of predicted *CYP2D6* IM and EM phenotypes using FFPEs, resulting mostly from *CYP2D6* deletions that cannot be determined from FFPEs, such misclassification could not alter the conclusions of the BIG1-98 investigation. In that investigation, in which the observed hazard ratio of recurrence was near 1.0 for *CYP2D6* PM and IM phenotypes vs EM phenotype, upwards of 75% misclassification would be necessary to

CYP2D6 score from WBCs	From FFPEs							From FFPELNs						
	PM	IM	IM	IM	EM	Total	PM	IM	IM	IM	EM	Total		
	ND	0	0.5	1	1.5	2	ND	0	0.5	1	1.5	2	Total	
0	0	4	0	0	0	0	4	0	3	0	0	1	0	4
0.5	0	0	7	0	0	0	7	0	0	7	0	0	0	7
1	1	0	1	42	0	0	44	2	0	1	41	0	0	44
1.5	0	0	0	0	15	0	15	1	0	0	0	14	0	15
2	0	0	0	0	1	51	52	3	0	0	0	0	49	52
Total	1	4	8	42	16	51	122	6	3	8	41	15	49	122
CYP2D6 score concordance		119/121 (98.3%; 95% CI = 94.2% to 99.8%)					114/117 (97.4%; 95% CI = 92.7% to 99.5%)							
agreement		$\kappa = 0.98$ (95% CI = 0.94 to 1.00)					$\kappa = 0.96$ (95% CI = 0.92 to 1.00)							
CYP2D6 phenotype concordance		120/121 (99.2%; 95% CI = 95.5 to 1.0%)					115/117 (98.3; 95% CI = 94.0% to 99.8%)							
agreement		$\kappa = 0.98$ (95% CI = 0.95 to 1.00)					$\kappa = 0.97$ (95% CI = 0.92 to 1.00)							

**Figure 1.** Concordance and agreement of *CYP2D6* scores and predicted phenotypes of 122 breast cancer patients' matched DNA samples from white blood cells (WBCs), formalin-fixed, paraffin-embedded tumors (FFPEs), and formalin-fixed, paraffin-embedded unaffected lymph nodes (FFPELNs). Genotypes could not be determined for one FFPE

and five FFPELNs because of low DNA quality/concentration and for one FFPELN for unknown reasons. Concordance and agreement exclude the one nondetermined (ND) FFPE and five/six ND FFPELN samples without adequate DNA quality/concentration for genotyping. EM = extensive metabolizer; IM = intermediate metabolizer; PM = poor metabolizer.

have obscured a true hazard ratio of 1.5 or greater. Although it is preferable to use DNA from WBCs for germline pharmacogenetic studies, in situations where the only source of DNA is FFPETs, these specimens are an appropriate alternative for *CYP2D6* genotyping and their corresponding *CYP2D6* phenotype determination.

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