

Does CYP2D6 Genotyping have a Role in Guiding Tamoxifen Therapy?

Kinan Mokbel and Kefah Mokbel*

The London Breast Institute, The Princess Grace Hospital, London, UK

*Corresponding author: Kefah Mokbel, The London Breast Institute, The Princess Grace Hospital, London, UK, Tel: 0044 (0)207 9082040; E-mail: kefahmokbel@hotmail.com

Received Date: September 28, 2017; Accepted Date: October 12, 2017; Published Date: October 17, 2017

Citation: Mokbel K, Mokbel K (2017) Does CYP2D6 Genotyping have a Role in Guiding Tamoxifen Therapy? Biochem Mol Biol J Vol. 3: No. 3:13.

Abstract

Inter-individual variability to drug response is a major concern in the field of oncology. In the era of "targeted" therapy, optimal treatment outcomes can be achieved by more individualized initial dosing and/or alternative treatment according to the patient's genetic make-up. Although several genes are responsible for the inter-individual variability in drug metabolism and response, the Cytochrome P450 enzymes are the most widely validated and clinically utilized. Pharmacogenetic testing of CYP2D6 alleles was the first FDA approved test due to its involvement in the metabolism of a wide range of drugs such as the anti-cancer drug tamoxifen (Nolvadex®), to which clinical response varies widely among patients.

Identifying determinants and predictors of the variable response or non-response to tamoxifen can facilitate therapeutic dose measurement or even determine the choice of alternative agents (e.g. aromatase inhibitors). Several clinical trials have shown that genetic variants associated with slower metabolism of tamoxifen may lead to lower than expected blood levels of its pharmacologically active metabolites and thus shorter recurrence-free survival. CYP2D6 genotyping can be clinically useful for selecting adjuvant therapy, improving the clinical outcome of tamoxifen and potentially reducing overall costs of treatment. However, CYP2D6 phenotype-based recommendations for tamoxifen have not been developed and guidelines linking the CYP2D6 status to personalized oncologic care do not exist and therefore clinicians' uptake of the testing has remained very low. Large prospective randomized clinical trials are required to assess whether CYP2D6 genotype can robustly predict treatment outcome of tamoxifen and improve overall survival.

Keywords: Pharmacogenetic; Breast cancers; Anti-oestrogenic

Introduction

Tamoxifen, a selective oestrogen receptor modulator, has been the mainstay for the therapy and prevention of

recurrence in pre-menopausal women with oestrogen receptor-positive breast cancers for almost 40 years. Tamoxifen decreases the risk of relapse by half and the mortality rate by nearly a third in patients with ER (+) breast cancer which count for approximately 80% of all breast cancers [1-3]. While aromatase inhibitors are more effective for treating post-menopausal patients with ER (+) breast cancer, tamoxifen was approved by the FDA for metastatic breast cancer and as an alternative to aromatase inhibitors for the chemoprevention in post-menopausal women [4-6]. Hence, tamoxifen remains the gold standard for ER (+) breast cancer. However, tamoxifen efficacy varies widely, many breast cancer patients with adjuvant tamoxifen remain free of recurrence but 30-50% suffer relapse [7-10].

Literature Review

Metabolism of tamoxifen

The pro-drug, tamoxifen, is extensively metabolised by CYP2D6 into therapeutically-active moieties, 4-hydroxy-tamoxifen and endoxifen; their affinity for the oestrogen-receptors is ~100-fold greater than tamoxifen and their anti-oestrogenic potency in suppressing ER-dependent cell proliferation is 30-100-fold stronger than tamoxifen [6,9,11-15].

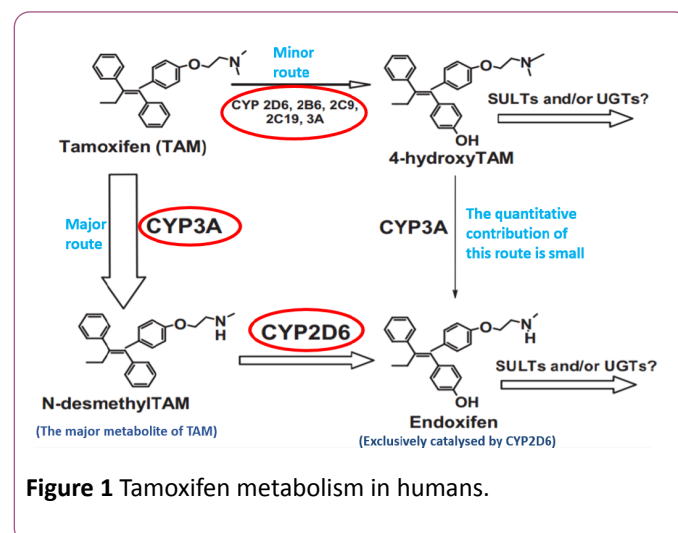


Figure 1 Tamoxifen metabolism in humans.

Although factors underlying the ineffectiveness of tamoxifen are yet to be fully defined, these active metabolites play a key

role in the high degree of interindividual differences in response to tamoxifen therapy [7-10].

Tamoxifen is metabolised via two routes; 4-hydroxylation and N-demethylation which account for ~7% and ~92% of tamoxifen metabolism, respectively. The 4-hydroxylation pathway, which is catalysed primarily via CYP2D6, leads immediately to the formation of 4-hydroxy-tamoxifen. N-demethylation results in the formation of N-desmethyl-tamoxifen and is catalysed mainly via CYP3A4/5, and is followed by CYP2D6-mediated secondary metabolism to endoxifen (Figure 1). However, endoxifen can be formed from both N-desmethyl-tamoxifen and 4-hydroxy-tamoxifen and it

has been found to play a more significant antiproliferative role than 4-hydroxy-tamoxifen in breast cancer [13,16-19]. Thus, it has been hypothesised that single nucleotide polymorphisms (SNPs) in the highly-polymorphic CYP2D6 can affect tamoxifen biotransformation and therefore may have the potential to predict the clinical outcome of tamoxifen therapy [9,19,20].

Cyp2d6 genotypes and predicted phenotypes

More than 100 variants, including more than 20 poor metaboliser alleles, with significant interethnic differences were identified [16,21,22] (Table 1).

Table 1 Allelic frequencies of CYP2D6 variant in selected population.

CYP2D6 variant	Predicted enzymatic function	Caucasian (Europe)	Caucasian (USA)	African-American
*1	Normal	33-36%	27-40%	29-35%
*2 (35%)	Normal	22-33%	26-34%	18-27%
*3	Deficient	1-4%	1-1.4%	<1%
*4	Deficient	12-23%	18-23%	6-9%
*5	Deficient	2-7%	2-4%	6-7%
*6	Deficient	1-1.4%	1%	<1%
*9	Decreased activity	0-2.6%	2-3%	<1%
*10	Decreased activity	1.4-2%	2-8%	34%
*17	Decreased activity	<1%	<1%	15-26%
*41	Decreased activity	20%	-	-

Note: The most clinically significant variants are circled in red

CYP2D6 different genotypes have been associated with four predicted phenotypes based on their CYP2D6 enzymatic

function: poor (PM), intermediate (IM), extensive (EM) and ultra-rapid (UM) metabolizers (Table 2).

Table 2 CYP2D6 enzymatic function.

Metabolizer status	CYP2D6-derived genotypes	Illepected effect
Ultra-rapid Metabolizer (UM)	Carrying more than two copies of normal functional alleles	Higher than expected concentrations of Tamoxifen metabolites at usual doses, possibly adverse reactions
Extensive metabolizers (EM)	Carrying two copies of "normal" function alleles	Normal
Intermediate metabolizers (IM)	Carrying two reduced function alleles or one functional wild-type and one reduced function allele	Response between those of EMs and PMs
Poor metabolizers (PM)	Carrying only no-or low-function alleles/Homozygous or compound heterozygous for null alleles	Possibly non- response and increased risk for breast cancer recurrence

Evidence

The widely-accepted criteria used to evaluate pharmacogenetic tests are: analytical validity, clinical validity and clinical utility [23].

Analytic validity

The heterogeneity at the CYP2D6 locus and the high homology of CYP2D6 locus with its pseudogenes render

analyzing CYP2D6 using the widely available short-read "next-generation" sequencing platforms to identify duplicated alleles immensely challenging. Nevertheless, clinical "next-generation" sequencing and genotyping pipelines have been developed and validated for CYP2D6 pharmacogenetic testing. For example, microarray technology-based genotyping for common CYP2D6 alleles can be optimal with sensitivity and specificity up to 100% [6,24].

Clinical validity

While comparative double-blinded trials showed no correlation between tamoxifen plasma concentrations and clinical response in women treated with tamoxifen [25], plasma levels of endoxifen in those with reduced CYP2D6 activity were found to be considerably low in comparison with those whose CYP2D6 activity is normal [6,16,17,26]. However, since tamoxifen efficacy is dependent upon multiple factors, one of which may be endoxifen plasma level, the variability in clinical outcome of tamoxifen therapy cannot always be explained by CYP2D6 genotype. Furthermore, the precise clinical role of endoxifen in the overall efficacy of tamoxifen is debatable. Endoxifen plasma concentrations cannot be regarded as a surrogate outcome for clinical response and the association between endoxifen concentrations and breast cancer outcomes should be confirmed [27]. Some drug models suggest that both tamoxifen and endoxifen overwhelm oestrogen receptors and therefore small concentrations of endoxifen may be needed to block oestrogen receptors suggesting that variations in endoxifen plasma concentrations may not affect this mechanism [28]. However, endoxifen and other tamoxifen metabolites were proposed to have different mechanisms in modulating hormone receptors [29]. The inter-patient variability of tamoxifen metabolism and clinical outcomes in breast cancer may also result from genetic variation in enzymes involved in tamoxifen metabolism such as CYP3A, or resistance to chronic tamoxifen treatment due to activation of somatic mutations in the estrogenic receptor (ESR1) [2,30,31].

Various studies regarding treatment outcomes in the adjuvant and/or metastatic settings have reported that those with genetically impaired CYP2D6 appear to have experienced significantly higher rate of breast cancer recurrence and mortality than those with normal CYP2D6 enzymatic activity [10,20,32-38], suggesting that variants associated with reduced function alleles (e.g. *4/*10/*41) are robust predictors of tamoxifen's antitumoral efficacy [10,20,34,37]. Moreover, findings from a prospective study found that CYP2D6 polymorphisms were strongly associated with Ki-67 response, implying that CYP2D6 genetic variation is an important predictor for efficacy of tamoxifen in women with breast cancer [38]. However, Ki-67 response has not yet been validated as a surrogate marker for tamoxifen efficacy.

Most of these studies were retrospective [33,34,39] and therefore liable to substandard documentation. The prospective studies [20,32], however, were also retrospective in nature since CYP2D6 genotyping has been performed after the relapse had eventuated. Nonparametric analyses and substantial overlaps in the patients' data were also reported in the following studies [10,33-35]. Nevertheless, two prospective yet small studies [36,38] and two large trials ($n > 1300$) [35,40] have reported statistically strong correlation between CYP2D6 genotype and clinical outcome in patients with breast cancer taking tamoxifen. Although some studies assessed overall survival outcomes [34,39], the trials were mostly non-randomized and failed to show a statistically significant correlation between overall survival and CYP2D6

genotype and whether this association varies by therapy type. In the metastatic setting, however, a significant association was observed but the study was small and the evidence was equivocal and limited [20]. The small sample size and the low coverage of alleles genotyped in most of these studies have diminished the reliability of the tests and reduced the ability to identify false positive results. The insufficient allelic coverage can cause patient misclassification. However, it can be argued that a clear bias toward positive results was observed with higher allelic coverage [41]. Furthermore, the inclusion of ER/PR-negative patients, who do not respond to tamoxifen, might have resulted in underestimation of the impact of genotype on clinical outcome and potentially false negative results in some studies [28,41]. These studies have also assumed that CYP2D6 genotype has no impact on clinical outcomes in women with breast cancer who are not taking tamoxifen. The apparent disparity in the ethnic composition, sample size, study design, methodologies, therapeutic regimens, adherence to treatment, co-treatments with CYP2D6 inhibitors and the inconsistent inclusion criteria of the majority of these trials can partly explain the controversial results and render comparison difficult. This has been further complicated by the inconsistent genotype-phenotype assignment and extensive heterogeneity in definition of outcome, as many measured the recurrence or disease-free survival but only a very few assessed the overall survival. More importantly, none of these studies has suggested detailed therapeutic paradigms, or addressed the potential consequences of CYP2D6 testing-based guidance on individuals with phenotypes determined incorrectly.

In contrast, two studies ($n=226$ and $n=677$) reported conflicting findings and demonstrated significantly reverse association between clinical outcome of tamoxifen and low activity CYP2D6 genotype [42,43]. It can, however, be argued that using a high dose of tamoxifen in these trials [42,43] might have normalized the low CYP2D6 activity in PMs and IMs. Analyses from two of the most influential large prospective randomized studies ($n=1243$ and $n=588$), BIG 1-98 and ATAC, found a near-null correlation between CYP2D6 genotype and breast cancer prognosis in tamoxifen-treated women, and provided statistically strong evidence of the opposite [7,44]. However, this null association was also unexplainably observed in the patients receiving tamoxifen in combination with other anti-tumour agents [45]. It has been suggested that the DNA extracted from formalin-fixed paraffin-embedded tumour tissues used in these studies instead of germline DNA [7,44] has potentially led to erroneous genotyping due to the presence of loss of heterozygosity (LOH) at the unstable CYP2D6 locus in breast tumour DNA. This bias was detected by the massive deviation from Hardy-Weinberg equilibrium (i.e. expected versus observed allelic frequencies) [46-49]. However, departure from Hardy-Weinberg equilibrium has not been reported in several trials using DNA derived from breast tumour-infiltrated tissues [50]. Even though germline DNA was used or blood samples with (LOH) were excluded, no positive association between genetic variations of CYP2D6 and tamoxifen efficacy was observed [51-53]. Therefore, results from the abovementioned studies [7,44] can be considered

valid and should be included in evaluating the value of CYP2D6 genotyping in tamoxifen therapy [38,50,54-56].

To date, there are no standards to validate quality of pharmacogenetic association. "Validation of a true pharmacogenetic association, demonstration of so-called 'clinical validity', should be conducted in prospective clinical studies that are larger, have more homogeneous patients and treatment and systematically collect outcomes data" [31].

Clinical utility

Even though it has been recognized that there are certain circumstances in which CYP2D6 genotyping can be helpful for tamoxifen dose adjustment or consideration of alternative treatment [57], CYP2D6 testing is not currently recommended for women for whom tamoxifen treatment is being considered.

Several studies provided evidence that doubling the tamoxifen dose to 40mg/day, which may have normalized impaired metabolism, was clearly associated with an increased concentration of endoxifen in IMs [27,42,43,58,59]. Nevertheless, endoxifen plasma levels remained considerably low in PMs taking 40 mg/day [27] and several studies showed that CYP2D6 metabolizer status was not a reliable predictor of individualizing tamoxifen optimal dose [60]. Recently, it has been, however, found that adjusted doses of tamoxifen to 80 and 40 mg for poor and intermediate CYP2D6 metabolizers, respectively, had achieved therapeutic endoxifen levels in approximately 90% of patients. However, inter-patient variability remained [61-63]. Thus, it is still debatable whether patients benefit from the dose escalation of tamoxifen. Interestingly, recent data showed that the PM status did not affect the clinical outcomes in majority of the patients [64]. Furthermore, studies showed that CYP2D6 genotype alone can only partly explain the interindividual variability among patients treated with tamoxifen and that tamoxifen treatment cannot be individualized only by CYP2D6 genotype suggesting other genes involved in the metabolism of tamoxifen should be considered [30,65-69]. Therefore, a definitive exposure-response effect remains debated and additional research is needed to determine whether CYP2D6 PMs phenotypes or even patients with low endoxifen plasma levels will achieve better clinical outcomes with increased dose of tamoxifen.

In spite of the fact that CYP2D6 genotyping test for tamoxifen treatment might be appropriate to optimize treatment outcome in specific patients [70], at present, it is still not generally recommended. The analysis of the existing literature and current evidence seem to be insufficient to justify the clinical utilization of CYP2D6 pharmacogenetic test to effectively guide dose and decision making in women with breast cancer for whom tamoxifen is being considered. Hence, it seems to be immensely challenging to reach consensus whether tamoxifen treatment should be based on the CYP2D6 testing and whether CYP2D6 genotyping approach is reliable and accurate in predicting treatment outcomes. For reliable implementation of CYP2D6 genotyping, large prospective studies of uniformly-treated women which establish evidence that the predictive capability of CYP2D6 testing exceeds the current approaches are required. Strategies such as

therapeutic drug monitoring (TDM) and approaches based on phenotyping or direct measurements of endoxifen concentrations or even more recently administering chemical compounds that are unaffected by CYP2D6 metabolism such as Z-endoxifen have proved to be more useful and effective than CYP2D6 genotyping to individualize tamoxifen therapy. However, their value must be validated in prospective clinical studies as well [63,70-73].

Discussion

Recommendations and clinical guidelines

American Society of Clinical Oncology clinical practice (ASCO) guidelines and the National Comprehensive Cancer Network (NCCN) recommendations: Because of the controversial findings from the two above-mentioned big studies, BIG 1-98 and ATAC trial, in which no relationship was found between the CYP2D6 genotype and breast cancer recurrence, the committee of the American Society of Clinical Oncology clinical practice (ASCO) and the National Comprehensive Cancer Network (NCCN) do not recommend CYP2D6 genotype testing to personalize tamoxifen therapy and therefore CYP2D6 allelic status should not be used to guide adjuvant endocrine therapy. Although no definitive conclusion was made with regard to the interaction between CYP2D6 inhibitors and tamoxifen, NCCN and ASCO recommend that concomitant use of CYP2D6 inhibitors and tamoxifen should be avoided due to drug-drug interactions [74-76].

The FDA recommendations: Initial findings from retrospective studies prompted the FDA Advisory Committee in 2006 to recommend re-labelling tamoxifen to state that PMs patients have a high incidence of recurrence in comparison with other genotypes [34,77]. Since then, several studies have reported conflicting and highly discordant results [7,44,70,78]. At present, the FDA does not recommend the pharmacogenetic testing for CYP2D6 and current FDA label of tamoxifen does not refer to CYP2D6 genotyping [79,80].

PharmaGKB and the Dutch pharmacogenetics working group recommendations: For CYP2D6 poor (PMs) and intermediate (IMs) metabolizers, alternative drugs are recommended for treating postmenopausal patients with breast cancer and concurrent use of CYP2D6 inhibitors with tamoxifen to be avoided [81].

Conclusion

As genotyping costs drop and CYP2D6 genotyping has the potential to guide hormone therapy, one may inevitably anticipate a wide adoption of the test in clinical setting. However, the existing evidence does not support a statistically strong correlation between variant genotypes of CYP2D6 and breast cancer outcomes. The discrepant and conflicting findings regarding the influence of CYP2D6 genotype on clinical outcomes with tamoxifen have caused perplexity among regulatory bodies and health professionals.

Even if the evidence was consistent for a strong association between CYP2D6 genotype and treatment outcomes, at best, reimbursement decisions by governmental or insurance schemes are based partly on evidence of cost-effectiveness. Therefore, the major challenges that are hindering the adoption of CYP2D6 genotyping include the relatively small proportion of non-response to tamoxifen, cost implications of testing and availability of alternative drugs that may exceed the benefits achieved by tamoxifen therapy. One could argue that it is unethical not to offer CYP2D6 pharmacogenetic screening prior to initiation of tamoxifen to women who might take it for 5 or 10 years, since it is known that certain women indeed will not respond to a standard dose of it. However, to achieve convincing evidence of clinical validity, large scale prospective randomized trials are warranted to robustly and quantitatively demonstrate that knowledge of the patient's CYP2D6 genotype can improve overall survival or reduce overall treatment cost in clinical practice. Further functional analysis is also required to elucidate the biological mechanisms underlying the metabolism and efficacy of tamoxifen and to further our understanding of the association between CYP2D6 phenotype and long-term clinical outcome.

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