Pharmacogenetics of Cytochrome P450 2D6: A Translational Medicine Perspective

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Abstract: With rapid scientific advances in the postgenomic era, pharmacogenomics has developed into an important area of translational medicine research. Pharmacogenetics studies the variability in drug response between different patient genotypes. By using patient genomic information, this bench-to-bedside research approach aims to develop more effective targeting of drug therapy in clinical practice. This will potentially reduce the risk of adverse drug reactions and avoid treatment failure. One of the important areas of pharmacogenetic variability is in drug metabolism via the cytochrome P450 (CYP) enzyme system. Cytochrome P450 2D6 (CYP2D6) metabolises up to 30% of commonly used drugs but this enzyme displays extensive genetic polymorphism. The approval of *CYP2D6* genotype testing by the FDA in 2005 has put this enzyme at the cutting-edge of research into personalised medicine. The effects of *CYP2D6* genetic polymorphism on tamoxifen metabolism and clinical outcomes in breast cancer patients is currently an important area of translational research. This review article covers recent progress in CYP2D6 pharmacogenomics and its applications to translational medicine.

Keywords: Cytochrome P450 2D6, pharmacogenomics, translational medicine.

INTRODUCTION

The elucidation of the human genome sequence has led to important advances in pharmacogenomics and translational medicine research [1-3]. Genetic factors can account for up to 30% in differences to drug response between patients [4]. These differences in patient genetic profiles can lead to an alteration in drug effect, with potential risks of drug toxicity or treatment failure [5]. Pharmacogenetics is the study of this interindividual patient variability to drug response and efficacy. Current clinical research is focusing on patient genotype testing and using this genetic information to provide more effective therapeutic targeting in clinical practice [6, 7].

The most widely studied group of proteins displaying pharmacogenetic variability are the drug metabolising enzymes and specifically, the cytochrome P450s (CYP). These enzymes are involved in the Phase I metabolism reactions, mainly resulting in drug substrate oxidation. Genetic polymorphism affecting the CYP enzymes can result in altered drug metabolism and efficacy [8]. Cytochrome P450 2D6 (CYP2D6) is one of the most extensively studied metabolic enzymes [9].

MOLECULAR MECHANISM OF CYP2D6

CYP2D6 functions as a mono-oxygenase enzyme and is predominantly found within the hepatic microsomes. It metabolises up to 30% of commonly used medications. Important drug classes metabolised by this enzyme include antidepressants, beta-blockers and analgesics. The drug substrates are mainly lipophilic bases with a protonated amine group and an aromatic ring [10]. The elucidation of the CYP2D6 crystal structure and the approval of *CYP2D6* genotype testing by the FDA in 2005 has put this enzyme at the cutting-edge of translational medicine research [11, 12].

The CYP2D6 enzyme is a 479 amino acid protein, which contains a heme group**;** Protein Data Bank ID: 2f9q [13]. CYP2D6 has a well-defined active site structure, which is located above the heme group. The amino acid residues that have been implicated in substrate recognition and binding are Asp301, Glu216, Phe483 and Phe120; Fig. (**1**). Both Asp301 and Glu216 play an important role in substrate binding [14]. The Phe483 and Phe120 residues control the alignment of the substrate molecule within the active site. CYP2D6 main function is drug substrate oxidation, *via* electron transfer initiated by substrate interaction with the heme-oxygen complex; Fig. (**2**). The catalytic reaction involves the insertion of one oxygen atom into the substrate molecule and the second oxygen atom is converted into water.

The electron source is provided by NADPH-cytochrome P450 reductase, a flavoenzyme that contains one molecule of each of the coenzymes, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) [15]. The FAD accepts electrons from the NADPH and the reduced species, $FADH^-$ transfers the electrons to FMN [16]. The $FMNH^-$ then donates the electron to the heme complex, reducing the ferric ion (Fe^{3+}) to the ferrous state (Fe^{2+}). The CYP reductase binding region is found in the C-terminal region of CYP2D6. This region predominantly contains basic residues that bind to CYP reductase *via* salt bridges, with Arg-440 being an important binding group [17].

The oxidation reaction is initiated by the activation of an oxygen molecule by the ferrous cation (Fe^{2+}). This

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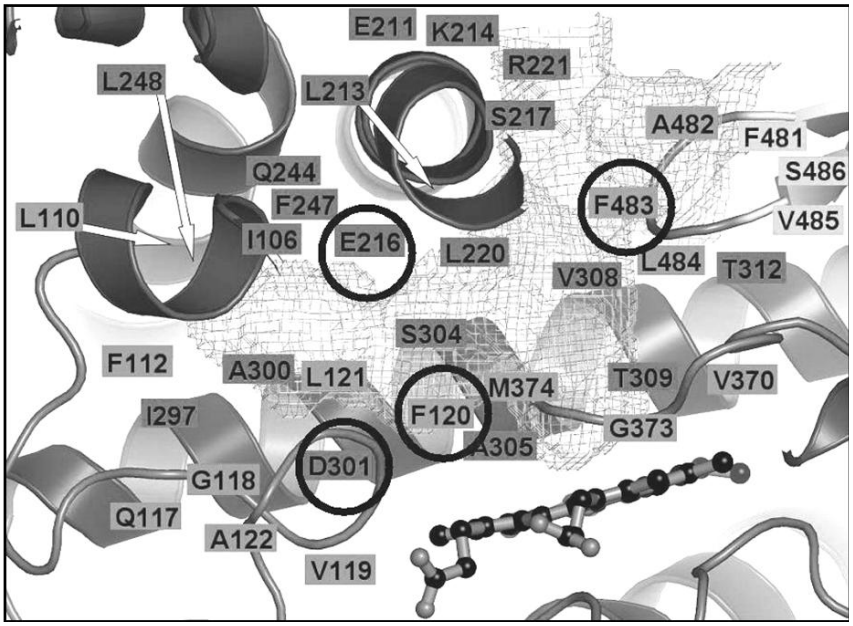

Fig. (1). CYP2D6 active site structure.

D301, E216, F120 and F483 are the important substrate recognition and binding residues. (Copyright: Rowland, P.; *et al. J. Biol. Chem.*, **2006**, *281*, 7614-22 [13]).

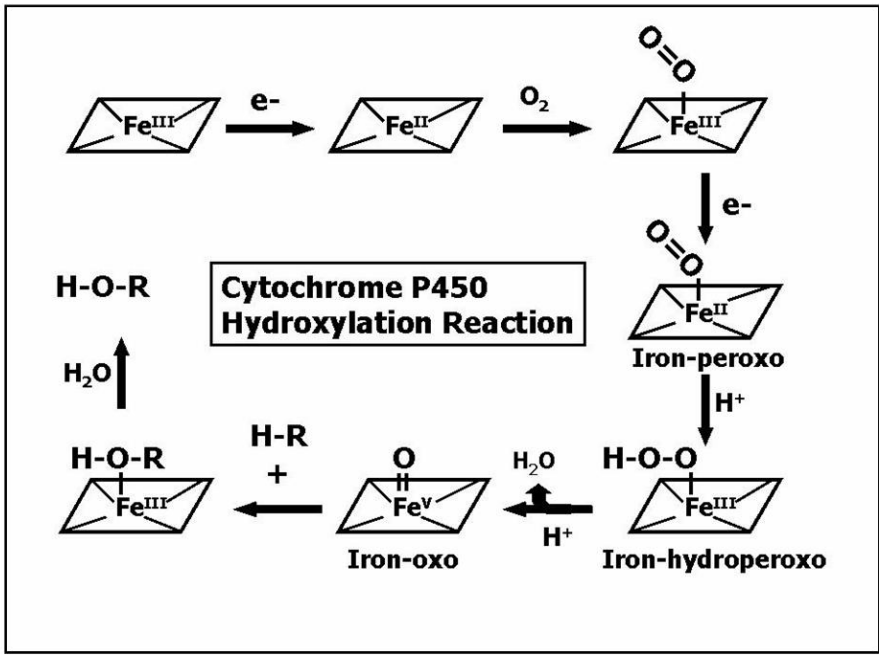

Fig. (2). Cytochrome P450 Catalytic Mechanism.

CYP catalysis involves the insertion of one oxygen atom into the drug substrate molecule (**H-R**) and the second oxygen atom is converted into water. The two main reactive iron-oxygen species involved in the catalytic reaction are the electrophilic iron-hydroperoxo and iron-oxo complexes.

hydroxylation reaction occurs at a distance of 5 - 7Å from the substrate molecule nitrogen atom. This produces an activated iron-oxygen species which reacts with the drug substrates through a variety of mechanisms [18]. The two main reactive iron-oxygen species involved in the catalytic reaction are the electrophilic iron-hydroperoxo and iron-oxo complexes [19]. The iron-hydroperoxo species is formed by protonation of the dioxygen-ferrous complex, iron-peroxo, Fig. (**2**). The iron-oxo species results from cleavage of the O-O bond. The postulated reaction mechanism involves free radical intermediates, although this still remains controversial [20].

GENETIC ASPECTS OF CYP2D6

CYP2D6 displays extensive genetic polymorphism that influences enzyme expression and function. The gene

encoding CYP2D6 is located on chromosome 22 (q13.1). Over 100 allelic variants of the *CYP2D6* gene have now been identified (*CYP2D6* allele nomenclature: www.cypalleles.ki.se/cyp2d6.htm). The enzyme metabolic activity also shows ethnogeographic variation, with differences between Caucasian, Oriental and Afro-Caribbean populations [9].

The three major allelic variants, which are found in the Caucasian population are *CYP2D6*3*, *CYP2D6*4* and *CYP2D6*5* (Table **1**). All three variants are associated with poor metaboliser (PM) phenotypes, with *CYP2D6*4* being the most frequent allele (20%) [9, 21]. The most commonly found allelic variant in the Oriental population is *CYP2D6*10* allele and this is associated with an intermediate metaboliser (IM) phenotype [22]. This allele results in the production of an unstable enzyme, caused by a double amino acid substitution (Table **1**) [23]. The most commonly found allele in the African population is *CYP2D6*17* [24]. It is also associated with an IM phenotype, resulting in reduced catalytic activity caused by a triple amino acid substitution (Table **1**) [23]. The ultra-rapid metaboliser (UM) phenotype is associated with *CYP2D6* gene multiplication and enzyme over-expression. This has been most commonly associated with the Ethiopian and Middle Eastern populations, with up to 30% allelic frequency [25, 26]. Due to this extensive genetic polymorphism displayed by *CYP2D6*, the role of genotype and phenotype testing for this enzyme has become an important area of translational medicine and personalised medicine research [8].

The FDA approved *CYP2D6* genotype testing (Amplichip® CYP450) for clinical use in 2005 [27]. The CYP AmpliChip® test involves the identification of a defined genetic mutation in the *CYP2D6* and *CYP2C19* genes, which results in the expression of a specific drug metabolism phenotype. The genotype test is based on microarray technology, which allows multiple gene expression analysis. The test screens for susceptible patient genotypes and will potentially allow tailoring of drug therapy in an attempt to reduce adverse drug reactions (ADRs) and avoid treatment failure [28]. It classifies patients into the four CYP2D6 phenotypes (PM, IM, EM and UM) by testing for 33 known polymorphisms. Newer microarray technology and PCR methods for CYP genotyping are continuing to be developed [29, 30].

CLINICAL STUDIES OF CYP2D6 PHARMACO-GENETICS

Clinical studies have shown a potential increased risk of ADRs or treatment failure associated with different *CYP2D6* allelic variants [34]. Important drug classes which are metabolised by CYP2D6 and show pharmacogenetic variability are the antidepressants, antipsychotics, analgesics and beta-blockers [28, 34]. Recently, there has been a lot of interest in the effects of *CYP2D6* genetic polymorphism on tamoxifen metabolism and clinical outcomes in breast cancer patients [35, 36].

Tamoxifen, Fig. (**3**), is an important drug used in the treatment of breast cancer and metabolised by CYP2D6. Pharmacogenetic variability of this enzyme has been associated with altered tamoxifen treatment response and patient prognosis [37, 38]. Tamoxifen is metabolised into the more active metabolites, endoxifen and 4-hydroxytamoxifen, Fig. (**3**) [39]. Both of these metabolites have higher affinity for the oestrogen receptor compared to tamoxifen [40]. The major metabolite of tamoxifen is *N*-desmethyltamoxifen, which is produced *via* CYP3A4/5. This is then metabolised by CYP2D6 to endoxifen [41]. CYP2D6 also plays a minor role in metabolising tamoxifen to 4-hydroxytamoxifen, which is then metabolised into endoxifen *via* CYP3A4/5. Patients with CYP2D6 PM/IM phenotype status have been shown to respond less well to tamoxifen treatment due to reduced production of these more active metabolites [42-44]. This has been associated with adverse clinical outcomes in these patients [37, 42].

Clinical studies have shown variability in disease progression relating to *CYP2D6* genotype status, in breast cancer patients treated with tamoxifen. In Oriental patients,

Table 1. Common Allelic Variants of *CYP2D6*

***CYP2D6* Variant Allele**	**Mutation**	**Enzyme Activity**	**References**
*CYP2D6*2xN*	Gene duplication/ amplification	Increased activity (UM) (10- 30% allelic frequency in Ethiopian/Middle Eastern populations)	Aklillu *et al.* 1996 [25] McLellan *et al*. 1997 [26]
*CYP2D6*3*	Frameshift deletion Non-functional allele	Inactive enzyme (PM) (1- 3% allelic frequency in Caucasian population)	Sachse *et al.* 1997 [31]
*CYP2D6*4*	Defective splicing Non-functional allele	Inactive enzyme (PM) (20- 25% allelic frequency in Caucasian population)	Mizutani, 2003 [21] Marez *et al.* 1997 [32]
*CYP2D6*5*	Gene deletion Non-functional allele	No enzyme (PM) (~ 5% allelic frequencyin general population)	Steen *et al.* 1995 [33] Mizutani, 2003 [21]
*CYP2D6*10*	Double amino acid substitution (P34S, S486T)	Reduced activity due to unstable enzyme (IM) (~ 50% allelic frequency in Oriental population)	Lee *et al.* 2006 [22] Shen *et al.* 2007 [23]
*CYP2D6*17*	Triple amino acid substitution (T107I, R296C, S486T)	Reduced activity due to altered substrate affinity (IM) (~ 30% allelic frequency in African population)	Dandara *et al.* 2001 [24] Shen *et al.* 2007 [23]

those with the *CYP2D6*10* genotype have been shown to have poorer clinical outcomes after treatment with tamoxifen. In a Japanese study, patients with the *CYP2D6*10* allelic variant had higher breast cancer recurrence rates and shorter disease free periods [37]. Similar results were found in a Chinese population and the study also demonstrated lower concentrations of tamoxifen metabolites in *CYP2D6*10* genotypes [44]. Clinical studies in Caucasian breast cancer patients have shown that CYP2D6 PMs (*CYP2D6*4* and *CYP2D6*5* genotypes) treated with tamoxifen have higher cancer recurrence rates and mortality [38, 45]. There has therefore been increasing interest in the role *CYP2D6* genotype testing for drug treatment selection in breast cancer patients [46].

However, there have been conflicting results as some *CYP2D6* genotyping studies have shown no association between allelic variants and tamoxifen efficacy in breast cancer patients [47]. In a recent Japanese study there was found to be no correlation between *CYP2D6*10* genotype and prognosis in breast cancer patients treated with tamoxifen [48]. Similar results have been also shown for the *CYP2D6*4* genotype in a Swedish population [49]. Therefore more rigorous pharmacogenetic clinical studies are required to correlate pharmacokinetic data with clinical response and using larger sample sizes for the different patient phenotypes [50].

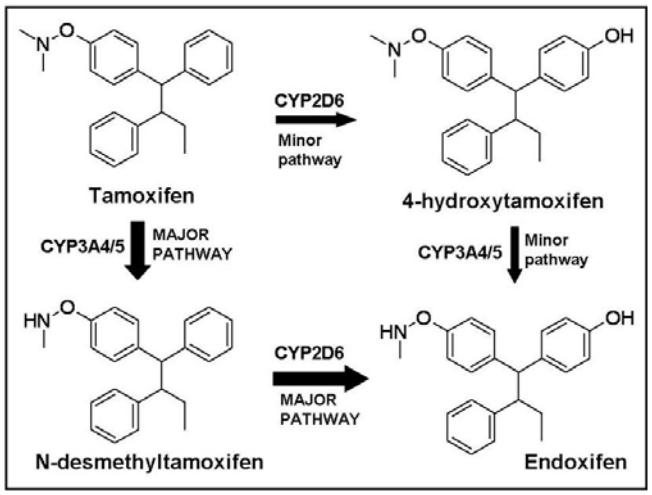

Fig. (3). Tamoxifen Metabolism and CYP2D6.

Tamoxifen is metabolised into the more active metabolites, 4-hydroxytamoxifen and endoxifen. The major metabolite of tamoxifen is *N*-desmethyltamoxifen, which is then metabolised *via* CYP2D6 to endoxifen. CYP2D6 also directly metabolises tamoxifen into 4-hydroxytamoxifen, which is then metabolised into endoxifen *via* CYP3A4/5.

Other important drug classes displaying altered clinical response relating to *CYP2D6* genotype status are the analgesics and beta-blockers. Tramadol is a commonly used painkiller, which is metabolised by CYP2D6 *via O*-demethylation into the more active metabolite *O*-desmethyltramadol, Fig. (**4**). A poor analgesic effect has been demonstrated in PM phenotypes treated with tramadol [51, 52]. On the other hand, UM phenotype patients have displayed an increased incidence of opioid related ADRs (nausea and respiratory depression) [53, 54]. *CYP2D6* genotype testing could therefore potentially help in the prevention of ADRs or treatment failure associated with tramadol use [55].

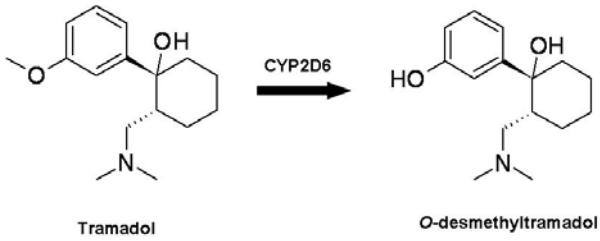

Fig. (4). Tramadol Metabolism and CYP2D6.

Tramadol is metabolised by CYP2D6 *via O*-demethylation into the more active metabolite *O*-desmethyltramadol.

Metoprolol is a β1-adrenoceptor antagonist, which is used in the treatment of ischaemic heart disease. Metoprolol is metabolised by CYP2D6 into its inactive metabolites, α-hydroxymetoprolol and *O*-desmethylmetoprolol. In experimental studies, CYP2D6 enzyme variants have shown reduced metoprolol metabolism [56]. Clinical studies of CYP2D6 PM phenotypes have demonstrated a potential increased risk of ADRs (hypotension and dizziness) associated with metoprolol treatment [57]. Genotype testing may therefore have a valuable role in beta-blocker therapy selection and further clinical studies are ongoing in this important area of cardiovascular medicine [58].

CONCLUSION

CYP2D6 pharmacogenomics represents an important area of translational medicine research. The effects of CYP drug metabolism has an important role in the drug discovery process and in lead candidate optimisation (ADME-Tox). CYP pharmacogenetics has also evolved into an important part of drug design and clinical trials [59, 60]. The elucidation of the CYP2D6 crystal structure may have a potential role in computational screening of suitable lead drug candidates [12]. The approval of *CYP2D6* genotype testing in clinical practice has been a major advance in personalised medicine research. This may facilitate drug treatment selection and avoidance of adverse drug reactions or treatment failure. The effects of *CYP2D6* genetic polymorphism on tamoxifen metabolism and clinical outcomes in breast cancer patients is currently a topical area of translational research.

Despite these important advances in pharmacogenomics, some barriers still remain in translating these research findings into clinical practice. A more rigorous evidence-base for *CYP2D6* genotype testing from larger pharmacogenomic clinical trials is required, to help develop standardised clinical guidelines for appropriate use of these tests [50]. Well-developed genomic services are also required for proper implementation of genotype testing and will require considerable education of healthcare professionals, in both the scientific and ethical aspects of pharmacogenomics [61]. With the current financial restrictions to most healthcare budgets, the challenges of funding these services also need to be considered. Results from ongoing clinical studies will provide further information into the cost-effectiveness of the different

genotype tests and the feasibility of integrating this into clinical practice [62].

ABBREVIATIONS

ADME-Tox = Absorption, Distribution, Metabolism, Elimination, Toxicology

ADR = Adverse Drug Reactions

CYP = Cytochrome P450

CYP2D6 = Cytochrome P450 2D6

EM = Extensive Metaboliser

FAD = Flavin adenine dinucleotide

FMN = Flavin mononucleotide

IM = Intermediate Metaboliser

NADPH = Nicotinamide adenine dinucleotide phospate

PM = Poor Metaboliser

PCR = Polymerase Chain Reaction

UM = Ultra-rapid Metaboliser

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