Pharmacogenetics: The Molecular Genetics of *CYP2D6* Dependent Drug Metabolism

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Abstract

Introduction: *Genetic variation of drug metabolising enzymes has been recognised as one of the major causes of the inter-individual variability to drug response. The vast majority of drugs are degraded via a small number of metabolic pathways, mainly by microsomal P-450 enzymes localised in the liver and, to a minor extent, in the small intestine. Of these,* **CYP3A4** *is the isozyme involved in the metabolism of most of the clinically useful drugs (50%). This is followed by* **CYP2D6** *(20%),* **CYP2C9** *and* **CYP2C19** *(15%). In addition, minor pathways are catalysed by* **CYP2E1, CYP1A2, CYP2A6** *and unidentified P-450s. Almost 40% of human P-450 dependent drug metabolism is carried out by genetically polymorphic enzymes. Polymorphisms generated by mutations in the genes for these enzymes cause quantitatively or qualitatively altered enzyme expression or activity through multiple molecular mechanisms. While* **CYP3A4** *genetic polymorphisms are just beginning to be unraveled, extensive studies on the* **CYP2D6** *gene over the last decade have identified at least 53 alleles. Of these, more than 20 of them are known to significantly alter the metabolism of* **CYP2D6** *substrates.* ***Methods:*** *This article reviews the information derived from various studies over the past decade and explains the molecular basis of functional differences in* **CYP2D6** *variants, especially with respect to inter-ethnic differences and their clinical implications.* ***Results:*** **CYP2D6** *activity ranges from complete absence to ultra-rapid metabolism. Large inter-individual and inter-ethnic variability exists in the activity of the enzyme, and consequently in the disposition of drugs undergoing oxidative metabolism.* ***Conclusions:*** *Pharmacokinetic differences resulting from these polymorphisms show potentially important clinical consequences.*

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Introduction

CYP2D6 is a cytochrome P450 isoenzyme that plays a role in the metabolism of a wide range of drugs, particularly cardiovascular and psychotropic agents. The *CYP2D6* locus at human chromosome 22 is a non-conserved locus with a heterogeneous number of genes and pseudogenes.[1] The wild-type locus is comprised of the active gene, *CYP2D6* and 2 highly homologous pseudogenes, *CYP2D7P* and *CYP2D8P* arranged in a 5' to 3' orientation on a contiguous region of about 45 KB. *CYP2D8P* contains multiple dele-tions and insertions, presenting a highly disrupted open reading frame. The instability of this area makes it a hot spot area for non-random chromosomal arrangements. Various point mutation or a combination of mutations, as well as re-arrangement of genes and pseudogenes of the *CYP2D6* cluster have resulted in *CYP2D6* alleles associated with absent, decreased or increased enzyme activity. As *CYP2D6* catalyses the oxidation of many clinically used drugs, variability in ability to metabolise these drugs has significant therapeutic consequences, ranging from increased risk for adverse effects at recommended doses for seriously impaired metabolism to therapeutic failures at normal dose for excess metabolic activity.

Genetic Polymorphisms

CYP2D6 is subjected to a well-defined genetic polymorphism, with a minority of the population[2,3] (<10%) lacking this enzyme as a result of a genetically transmitted defect in its expression. The *CYP2D6* polymorphism is expressed in 4 major phenotypes commonly termed poor metaboliser (PM), intermediate metaboliser (IM), rapid metaboliser (EM) and ultra-rapid metaboliser (UM), which occur with varying prevalence in different populations. The PM phenotype is usually associated with the possession

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of 2 alleles encoding an inactive enzyme, whereas the UM phenotype is related to an inherited amplification of functional *CYP2D6* genes.

Metabolic Probes

CYP2D6 phenotypes are traditionally identified by administration of a probe drug followed by estimation of parent to metabolite ratio in the urine. In normal or extensive metabolisers, the metabolites predominate, in slow metabolisers, the parent compound persists. However, the parent compounds may then be metabolised along other metabolic routes. Debrisoquine, an anti-hypertensive agent, was the original probe[4,5] that led to the discovery of the genetic deficiency of *CYP2D6*. It has, however, been withdrawn from registration in Singapore since 1996 because of the lack of clinical indications and thus, has limited availability for use in genetic characterisation.

Recently, however, it has been shown that the *CYP2D6* polymorphism can be demonstrated by using dextromethorphan as probe drug.[6,7] Dextromethorphan is an isomer of levorphanol, a codeine analog. Unlike codeine, dextromethorphan exhibits no addictive or analgesic properties at usual therapeutic doses. It is, however, a widely used centrally acting antitussive agent that has low toxicity. The availability and safety of this drug appear to make it an ideal test substance for establishing an individual's drug metabolism phenotype. Phenotyping is based upon the O-demethylation of dextromethorphan. Individuals who are poor metabolisers have lower amounts of the O-demethylated metabolite, dextrophan, in their urine than individuals who are extensive metabolisers. The ratio of 0.3 is generally used to distinguish poor metaboliser from the extensive metabolisers. This is based on a large Swiss study of 268 subjects where a bimodal distribution was obtained with a gap between ratios of 0.2 and 0.6.[8] The antimode of 0.3 between the EM and PM was determined by density function analysis.

Molecular Basis of Functional Differences in *CYP2D6* Variants

The genetic polymorphism at the *CYP2D6* locus potentially results in severely compromised metabolism of at least 25 drugs.[3] Defective alleles can be the result of gene deletions, gene conversions with related pseudogenes and single base mutations causing frameshift, missense, nonsense or splice-site mutations. The homozygous presence of such alleles leads to a total absence of active enzyme and an impaired ability to metabolise substrates specific for *CYP2D6*. On the contrary, replication of

Fig. 1. Major molecular mechanisms involved in altered CYP2D6 *dependent metabolism. Arrows represent transcription-translation pathways. Genetically altered pathways due to gene deletion, missense, nonsense, frameshift and splice-site mutations lead to altered (none, increased, decreased) metabolism. Examples of* CYP2D6 *alleles are listed under each category.*

functional *CYP2D6* gene causes ultra-rapid metabolism. Figure 1 shows the possible molecular mechanisms leading to complete absence, decreased or enhanced metabolic activity. Frameshift mutation resulting from a nucleotide deletion causes the ribosome to read a completely new set of codons downstream from the mutation. A completely different and often lethal phenotype is produced. Splicing removes part of the introns from a primary transcript of a gene. Recombination and crossing-over events produce variants with very different phenotypes ranging from a total lack of to excess enzyme activity.

Functionally Inactive Alleles

Several completely inactive alleles have been found for *CYP2D6*.[2,3] These include a single base deletion, A_{2549} in exon 5 with consequent reading frame disruption (*CYP2D6*3*), a point mutation $G_{1846}A$ at the consensus sequence of the 3' splice site of intron 3 (*CYP2D6*4*) causing a splicing defect, 2 different large deletions[9] of the *CYP2D6* gene (*CYP2D6*5*, 11.5 kb or 13 kb), a single nucleotide exchange in exon 6 ($A_{2935}C$) resulting in a his324pro change (*CYP2D6*7*), and a single base deletion T_{1707} in exon 3 (*CYP2D6*6*) with a consequent frame shift and a generation of a stop codon. *CYP2D6*5*, *CYP2D6*3* and *CYP2D6*4* are among the commonest defective alleles responsible for the PM phenotype.[2,3]

Alleles Causing Functionally Diminished or Altered Enzyme Activity

In addition to the defective alleles, there are also some alleles that cause diminished or altered drug metabolism. An example is the *CYP2D6*10* allele commonly found in Chinese and other Orientals.[10] A $C_{100}T$ mutation in exon 1 causes a $Pro_{34}Ser$ substitution in the proline-rich region near the NH_2 terminal. This region is highly conserved among microsomal P-450s and may function as a hinge between the hydrophobic membrane anchor and the globular heme-binding portion of the enzyme. This nucleotide substitution results in an enzyme product that exhibits impaired folding capacity, thereby reduced expression of functional enzyme. The presence of the *CYP2D6*10* alleles appears to translate to lesser activity as the metabolic ratio of debrisoquine shift towards a higher value.[11,12] Homozygous *CYP2D6*10* subjects were found to have smaller metabolic ratio than that of the poor metabolisers but larger than that of homozygous *CYP2D6*1*.[7] This

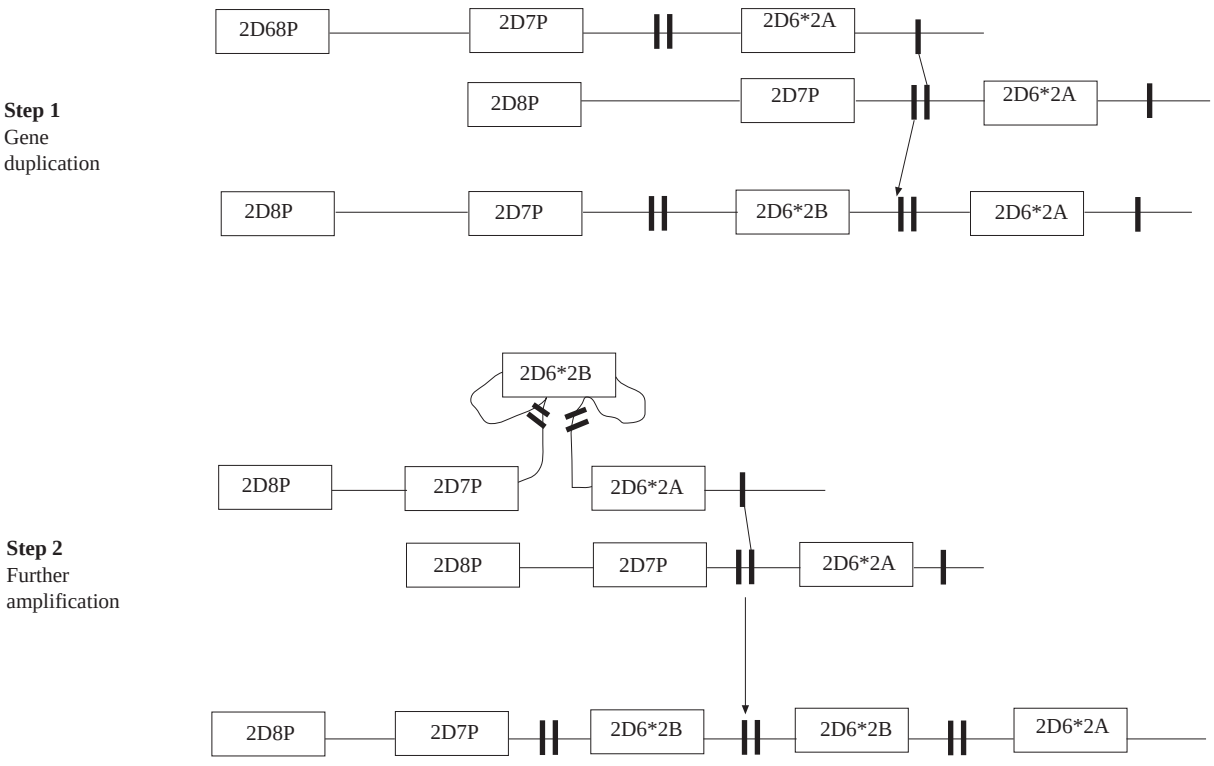

Fig. 2. The unequal-exchange model showing the formation of CYP2D6 *haplotypes with more than one copy of the* CYP2D6**2 allele. Step 1 shows unequal cross-over and formation of the 42kb Xba I haplotype. Step 2 shows positioning of the Alu repeats to create a loop for further amplification of the gene.

explains the lower capacity amongst Chinese to metabolise drug substrates of *CYP2D6* such as antidepressants and neuroleptics.

Alleles Causing Functionally Enhanced Metabolic Activity

On the other extreme of *CYP2D6* metabolism, a trait found in 2% to 7% of Caucasians and as high as 20% to 30% of Indians,[13] Ethiopians[14] and Saudi Arabians,[15] has been shown to cause greatly enhanced metabolism of substrates. The genetic basis of this phenomenon is gene duplication or amplification of functional active *CYP2D6* genes, resulting in higher expression of enzymes.[16] The mechanism behind this genetic event involves mainly one allelic variant, the *CYP2D6*2*, although involvement of other variants like *CYP2D6*1*, *CYP2D6*4* have been reported.

Tandem repeats of *CYP2D6* alleles with up to 5 copies can be formed by unequal recombination between 2 homologous but non-allelic sequences flanking the gene. The unequal cross-over is believed to have occurred at a specific breakpoint in the 3' flanking region of the *CYP2D6*2B* allele with a specific repetitive sequence or in the Alu sequences 3' of the *CYP2D7P* and the *CYP2D6* genes. The Alu repetitive sequences surrounded by long AT-rich regions are known recombinational hot spots. The unequal cross-over results in one locus having 2 genes in tandem and another locus with a deleted gene. Tandem repeats of up to 13 copies can result from unequal segregation and extra-chromosomal replication of acentric DNA. The Alu sequences surrounding the first duplication can act in cis and form hairpin loops to loop out the gene segment for further duplication during sister chromatid exchanges as illustrated in Figure 2. The multiple copies in the new cells are then arranged in sequence. The resultant UM phenotype is typically characterised by 2, 3, 4, 5 or 13 copies of the same allele. The clinical consequence of this gene arrangement is inability to achieve therapeutic drug levels at ordinary drug dosages due to ultra-rapid clearance.

Impact of *CYP2D6* Polymorphisms on *In vivo* Drug Metabolism

Individuals lacking functional *CYP2D6* alleles exhibit greater bioavailability, greater plasma concentrations, prolonged elimination half-lives, and possibly exaggerated pharmacologic response from standard doses of drugs that are metabolised by *CYP2D6* enzyme. They are at risk of developing potentially toxic concentrations of these drugs and may suffer from related hazardous drug interactions. Table I shows the impact of defective *CYP2D6* alleles on some common drug treatment. PMs metabolise selective *CYP2D6* substrates, particularly antidepressants and neuroleptics, at a much reduced rate. Several known defective *CYP2D6* genotypes have reliably predicted the clearances of antidepressants fluvoxamine, desipramine, fluoxetine, mexiletine and citalopram as well as neuroleptics perphenazine and zuclopenthixol. Adverse effects due to elevated drug plasma levels occur more frequently where drug clearance is primarily dependent on *CYP2D6*. PMs are also more likely to suffer from neuropathy after treatment with the anti-anginal, perhexiline. Adverse effects such as nausea, vomiting and arrhythmias may occur in PMs during treatment with dexfenfluramine, propafenone and mexiletine, respectively, as a consequent to elevated plasma drug concentrations. On the contrary, where pro-drugs requiring activation by *CYP2D6* are given to subjects lacking the enzyme activity, reduced therapeutic effectiveness occurs. For example, the analgesic effect of tramadol can be severely reduced in PMs.

TABLE I: IMPACT OF DEFECTIVE *CYP2D6* ALLELES ON SOME COMMON DRUG TREATMENTS

Decreased clearance	Adverse effects	Reduced prodrug activation
Tricyclic antidepressants	Cardiotoxicity	Tramadol
		Codeine
Haloperidol	Parkinsonism (?)	Ethymorphine
Anti-arrhythmic drugs	Arrhythmias	
Perphenazine		
Perhexiline	Neuropathy	
SSRIs	Nausea	
Zuclopenthixol		

SSRI: selective serotonin reuptake inhibitors

In individuals with multiple copies of the *CYP2D6* gene, ultra-rapid metabolism of substrate drugs makes it difficult to achieve therapeutic drug plasma levels with normal doses. Metabolic activity is apparently directly proportional to the number of functional gene copies carried by the subject. The gene-dosage effect has been clearly demonstrated in a nortriptyline clearance study.[17] Subjects with higher copies of the genes have an obvious higher metabolite formation rate. Extensive formation of morphine is known to occur in subjects with replicated alleles taking the prodrug codeine. Severe abdominal pain, a typical adverse effect of morphine, has been observed as a consequence of ultra-rapid activation of codeine to morphine.

Inter-ethnic Differences and Clinical Implications

CYP2D6 exhibits interesting ethnic differences in that, the frequency of poor metabolisers in Chinese, Koreans and Japanese population is less than 1%;[10,12] whereas, the frequency is much higher among Caucasians (5% to

TABLE II: *CYP2D6* ALLELES FREQUENCIES IN LOCAL ETHNIC GROUPS

	*CYP2D6*5*	*CYP2D6*10* $C_{100}T$ only	*CYP2D6*rep*	*CYP2D6*3*	*CYP2D6*4*	Total no.
	No. (frequency)	No. (frequency)	No. (frequency)	No. (frequency)	No. (frequency)	(alleles)
Chinese	14 (0.07)	92 (0.48)	7 (0.04)	0 (0)	2 (0.01)	192
Malays	14 (0.12)	44 (0.37)	6 (0.08)	–	–	120
Indians	8 (0.08)	20 (0.22)	24 (0.25)	–	–	96
Caucasians (reported frequency)	0.02-0.09	0.02-0.05	0.05-0.07	0.06-0.34	0.17-0.33	–

10%).[11,18,19] The splice site mutation $G_{1846}A$ (*CYP2D6*4*) and the deletion of the entire gene (*CYP2D6*5*) represent the most common defects in Caucasians. These inactivating mutations are rare in Asians. However, oriental EMs tend to have a lower metabolic activity than their Caucasian counterpart and this appears to be associated with the higher frequency of the *CYP2D6*10* alleles among them.[20] The diminished enzyme activity is due to a $Pro_{34}Ser$ mutation that affects the expression of the enzyme.

The frequency of *CYP2D6*10* alleles is about 0.47 in Chinese,[10] 0.45 in Japanese[11] and 0.51 in Koreans[8] but only 0.05 in Caucasians.[7] Therefore, a significant group of Orientals may be expected to possess "intermediate" *CYP2D6* activity. The clinical outcomes of the frequent distribution of the deficient *CYP2D6*10* allele in the Asian population and the resulting metabolic deficiency are evidenced by several examples. This includes the generally lower doses of neuroleptics required in Asians and higher plasma haloperidol and S-mianserin concentrations in Asian patients. Tsuyoshi and coworkers[12] have demonstrated that the AUC of venlafazine was 484% greater in homozygous *CYP2D6*10* as compared to homozygous *CYP2D6*1*. A similar conclusion was made with propanolol[21] and codeine[22] among Chinese subjects carrying the *CYP2D6*10* alleles.

On the other end of the metabolic scale, 2% to 7% of Caucasians,[16] 29% of Ethiopians,[14] 20% of Saudi Arabians[15] and 25% of Indians[13,23] carry duplicated or multi-duplicated *CYP2D6* genes that confer ultra-rapid metabolism. This often results in therapeutic failures in these subjects as only suboptimal plasma concentrations of drugs can be achieved at normal doses. As most drug dosing regimens are based on normal metabolic rate, a significant proportion of people in ethnic groups where the frequency of the *CYP2D6*rep* allele is high would require major dosage adjustments for effective treatment with *CYP2D6* metabolised drugs.

CYP2D6 Polymorphisms in the Local Population

CYP2D6 alleles distribution in 3 major local ethnic groups, namely, the Chinese, Malays and the Indians[13,23] has been recently reported. The breakdown of the alleles frequencies is reproduced in Table II. In the Chinese, *CYP2D6*3* was not detected while the frequencies of the inactive alleles *CYP2D6*4* and *CYP2D6*5* and the replicated allele *CYP2D6*rep* are relatively low (0.01, 0.07 and 0.04, respectively) as compared to existing Caucasians data.[6] The debilitating $C_{100}T$ mutation responsible for diminished enzyme activity in *CYP2D6*10* is most frequent (0.48). The Malays share a very similar pattern of allelic distribution. The frequencies of the inactive *CYP2D6*5* allele (0.12), the replicated *CYP2D6*rep* allele (0.08) and $C_{100}T$ mutation (0.37) are quite close to that in the Chinese. In contrast, the Indians have a markedly different alleles distribution with a frequency of as high as 0.25 for the *CYP2D6*rep* allele. Such high figures have only been reported for Ethiopians[14] and Saudi Arabians.[15]

Conclusions

Currently, major polymorphisms causing impaired *CYP2D6* dependent drug metabolism are well known.[24] Therapeutic failures and adverse drug reactions caused by defective alleles can be predicted to a large extent if the genotype of the subject is known. With the rapid progress in the development of gene chip technology, it may soon be quite feasible for all individuals to be genotyped with respect to critical genes essential for drug metabolism. Key genetic variations that could impact drug response and safety can thus be identified. This is of particular importance with respect to Asian populations. A significant proportion of individuals in our population carry the $C_{100}T$ mutation that results in lower predicted *CYP2D6* activities. This group of individuals is at a greater risk of excessive side effects from drug compounds that are metabolised mainly by the *CYP2D6* enzymes. This is because the standard dosing regimen for pharmaceutical agents are derived from studies conducted in predominantly EM Caucasian populations. This is expected as drug development takes place mainly in Europe and the United States. To ensure optimal dosing and therapeutic efficacy, it is thus essential that adequate studies be conducted in local populations where reduced enzyme activities are common. Dose ranging recommendations can thus be tailored for maximum therapeutic benefits and minimum adverse effects.

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