

GENETIC POLYMORPHISM OF *CYP2D6*

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SUMMARY

CYP2D6 is polymorphically distributed and is responsible for the metabolism of several clinically important drugs. It is also related to several pathophysiological conditions. Defect alleles, causing poor metaboliser (PM) phenotype and alleles with duplicated or multiduplicated active genes, causing ultra extensive metabolism (UEM) have been described. *CYP2D6* polymorphism exhibits pronounced interethnic variation. While initial observation and studies focused on population of Caucasian origin, later other populations also studied extensively. Differences in metabolism of drugs can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of pharmacologically active drug or metabolite. Knowledge of individual's *CYP2D6* status may be clinically and economically important and could provide the basis for a rational approach to drug prescription.

KEYWORDS Genetic polymorphism *CYP2D6* pharmacogenetics

INTRODUCTION

Genetic polymorphism is defined as the inheritance of a trait controlled by a single genetic locus with two alleles, in which the least common allele has a frequency of about 1% or greater¹. One of the most extensively studied genetic polymorphisms known to influence drug metabolism and response is the debrisoquine type (*CYP2D6*) oxidation polymorphism. The discovery of *CYP2D6* polymorphism created new interest in the role of pharmacogenetics in clinical pharmacology².

Genetic polymorphism has been linked to three classes of phenotypes based on the extent of drug metabolism. Extensive metabolism (EM) of a drug is characteristic of the normal population; poor metabolism (PM) is associated with accumulation of specific drug substrates and is typically an autosomal recessive trait requiring mutation and/or deletion of both alleles for phenotypic expression; and ultra extensive metabolism (UEM) results in increased drug metabolism and is an autosomal dominant trait arising from gene amplification³.

CYP

'CYP' is the abbreviation for cytochrome P-450, a subgroup of related enzymes or isoenzymes located in the endoplasmic reticulum and expressed mainly in the liver. It is also present in other organs, such as the intestine and the brain⁴. In mammals, most xenobiotics are metabolised *via* hepatic phase 1 metabolism by means of CYP monooxygenases⁵. Thirty or more different forms of these haem thiolate proteins have been characterized in humans³. The P450 superfamily is composed of families and sub-families of enzyme that are defined solely on the basis of their amino acid sequence similarities. With few exceptions, a P450 protein sequence from one family exhibits upto 40% resemblance to a P450 from other family. P450s within a single subfamily always share greater than 55% sequence similarity^{6,7}.

Evolution of *CYP2D6* polymorphism

Between 1975 and 1977 two groups independently discovered the genetic deficiency of debrisoquine⁸ and

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sparteine⁹ metabolism. The discovery of genetic polymorphism in the metabolism of the two prototype drugs was not the result of a planned strategy but rather an incidental observation. A dramatic event in a pharmacokinetic study prompted the initial search for a specific metabolic defect: the investigator, Dr. Smith, who was participating in a study on debrisoquine, a sympatholytic antihypertensive drug, had a much more pronounced hypotensive response than his colleagues, collapsing from a sub therapeutic dose. This was found to be due to impaired 4-hydroxylation of debrisoquine⁸.

Similarly in 1975, during the course of kinetic studies by Eichelbaun *et al* with a slow release preparation of sparteine, two subjects developed side effects such as diplopia, blurred vision, dizziness and headache. When analysing the plasma levels of sparteine in those subjects the reason for the development of side effects become evident. Compared to all the other subjects studied, their plasma levels were 3 to 4 times higher, although the same dose had been given to every subject⁹.

Family and population studies¹⁰ uncovered a genetic polymorphism and later work established that the two independently discovered defects in drug oxidation co-segregated in Caucasians (PM for sparteine exhibit impaired debrisoquine metabolism and vice versa) and the term sparteine/debrisoquine polymorphism was coined¹¹. However there are apparent exemptions to this rule. For instance, in a study in Ghana, the ability of Ghanaians to oxidise sparteine was independent of their capacity for debrisoquine oxidation¹².

Nomenclature

Guidelines on nomenclature for individual cytochrome P450 isoform have been internationally agreed upon and are regularly updated. Genes encoding the P450 enzyme are designated as CYP. Because of the diversity of the cytochrome family, a nomenclature system based on sequence identity has been developed to assist in unifying scientific efforts in this area and to provide a basis for nomenclature of newly recognized members of this gene superfamily. For example, *CYP2D6* is isoform 6 of subfamily D included in the 2 CYP family³.

In the past, *CYP2D6* alleles have been named arbitrarily using a single letter after the gene name,⁷ but

with increasing numbers of alleles being detected, this system is now inadequate. The general recommendation is that the gene and allele are separated by an asterisk. Specific alleles are named by Arabic numerals or a combination of Arabic numerals followed by a capitalized Latin letter. There are no spaces between gene, asterisk and allele and the entire gene-allele symbol is italicized (e.g. *CYP2D6*1A*)^{13,14}.

Since a number of *CYP2D6* alleles share common key mutations but differ with respect to other base changes, these should be given the same Arabic number (denoting their allele group) and distinguished by capitalized Latin letters (denoting the allele subgroups). For example, both *CYP2D6*4A* and *CYP2D6*4B* have the same mutation but differ by a single silent base substitution¹³.

Extra copies of an allele (duplicated or amplified) may exist in tandem; for example, the *CYP2D6L2* allele contains two copies of *CYP2D6L*. Here the entire arrangement of alleles should be referred to as *CYP2D6*2X2*. When duplication is not with the same subgroup, they are separated with a coma (e.g. *CYP2D6*10B,10C*)¹³.

A non-italicized form of the allele is used to name the protein with asterisk omitted and replaced by a single spacing e.g.: *CYP2D6* 1. Both alleles italicized and separated by slash to name the genotype designation (*CYP2D6*1/CYP2D6*4A*)^{13,14}.

For a review of the most recent nomenclature of *CYP2D6*, refer to Daly *et al*¹³ and Garte and Crosti¹⁴. This nomenclature system is also used for other P450 alleles like *CYP2A6*1*, *CYP2C9*2*, *CYP2C19*2* etc. Descriptions of the alleles as well as the nomenclature and relevant references are continuously updated at the new web page (<http://www.imm.ki.se/CYPalleles/>).

MOLECULAR GENETICS

The *CYP2D6* gene resides in the *CYP2D6-8* clusters on chromosome 22 in association with the *CYP2D7P* and *CYP2D8P* pseudogenes¹⁵. Defective alleles can be the result of gene deletion¹⁶, gene conversions with related pseudogenes and single base mutations¹⁷ causing frameshift, missense, nonsense or splice-site mutations^{18,19}. The homozygous presence of such alleles leads to a total absence of active enzyme and an impaired ability to metabolise

Table 1. Inhibitors of *CYP2D6*.

Ajmalicine	Ajmaline	Amitriptyline	Amesergide
Aprindine	Budipine	Bufuralol	Chloroquine
Chlorpromazine	Cimetidine	Cisthiothixene	Citalopram
Clomipramine	Clozepine	Desmethylinipramine	Diphenhydramine
Flecainide	Fluoxetine	Fluoxetine	Fluphenazine
Halofantrine	Haloperidol	Levomopromazine	Methadone
Meclobemide	Olanzapine	Oxprenolol	Paroxetine
Perazine	Perphenazine	Propofenone	Propranolol
Quinidine	Quinine	Ranitidine	Reboxetine
Resperidone	Sertraline	Terbinafine	Terfenadine
Thioridazine	Ticlopidine	Venlafaxine	Yohimbine

probe drugs specific for the drug-metabolizing enzyme. These subjects are classified as PM¹⁶⁻²⁰.

In addition to defective CYP genes, there are also alleles that cause diminished or altered drug metabolism. This results in enzyme products that exhibit impaired folding capacity and therefore the expression of the functional enzyme is severely diminished^{17,18}. Among extensive metabolisers, heterozygotes (one functional gene) have higher medium metabolic efficacy than those who are homozygous for the wild-type allele (two functional genes), but with pronounced overlap²¹⁻²³.

Another type of metabolism is known as ultra rapid metabolism and is caused by occurrence of duplicated, multiduplicated or amplified *CYP2D6* genes. At present, alleles with two, three, four, five and 13 gene copies in tandem have been reported and the number of individuals carrying multiple *CYP2D6* gene copies is highest in Ethiopia and Saudi Arabia, where up to one third of the population displays this phenotype¹⁸. In a Swedish family, a father, a daughter and a son were shown to have 12 copies of a functional *CYP2D6L* gene with one normal gene and showed extremely high *CYP2D6* activity²⁴.

Although clear criteria have not been formed to structurally assess whether a compound is metabolized by this enzyme, it is observed that most of *CYP2D6* metabolized substrates and inhibitors have a basic nitrogen and are oxidized at a site within 0.5-0.7nm

of this basic nitrogen. It may also have a flat lipophilic region and functional groups which have capacity for electrostatic interactions or the ability to form hydrogen bonds^{25,26}. The enzyme even shows stereoselectivity also. In extensive metabolizers, inactive R-metoprolol is metabolized faster than the active S-enantiomer whereas this metabolism is not stereoselective in poor metabolisers²⁷. Isoform selectivity of *CYP2D6* is observed in mianserin metabolism also²⁸.

INHIBITION AND INDUCTION OF *CYP2D6*

Quinidine is the most potent inhibitor ($k_i=0.03$) of *CYP2D6*²⁶. Quinine, which is a diastereoisomer of quinidine, is several hundred times less potent inhibitor than quinidine. However, quinidine is not a substrate of *CYP2D6*¹. Single oral dose of 200 mg quinidine sulphate is adequate to convert most extensive metabolisers to poor metabolisers²⁹. Fluoxetine³⁰⁻³², paroxetine³² and propofenone³³ are also potent inhibitors of *CYP2D6* with inhibition constant in the low nanomolar range. A list of inhibitors of *CYP2D6* is given in Table 1.

Unlike many members of the CYP enzyme family, the *CYP2D6* enzyme is not affected by classic enzyme inducers such as phenobarbitone³⁴. Rifampicin treatment has given only a 30% increase in clearance of sparteine, but metabolic ratio was not significantly changed³⁴. About 33% reduction in the metabolic ratio of debrisoquine has been observed in female EM using

contraceptives³⁵. During the menstrual cycle, insignificant decrease in debrisoquine metabolic ratio was observed during the luteal phase compared to preovulatory phase^{36, 37}. Heavy cigarette smoking and ovariectomy induced this enzyme activity but only to a minor extent¹. In contrast, there is evidence that pregnancy has a profound influence on *CYP2D6* activity. Marked increase in metabolism of metoprolol³⁸ and dextromethorphan³⁶ has been reported during pregnancy.

ASSESSMENT OF INDIVIDUAL *CYP2D6* ACTIVITY

The activity of *CYP2D6* enzyme can be assessed by means of phenotyping or genotyping³.

Phenotyping

Phenotyping requires intake of a probe drug; the metabolism of which is known to be solely dependent on *CYP2D6* enzyme. The excretion of parent compound and/or metabolite in urine allows to calculate the metabolic ratio, which is a measure of individual *CYP2D6* activity^{3, 11}.

In a typical phenotyping experiment, individuals were administered an oral dose of the probe drug usually at a subtherapeutic level, and urine was collected over a period of 8-12 hours. Total yield of parent compound and metabolites were determined and the metabolite/parent compound ratio, termed metabolic ratio (MR) was plotted as frequency distribution histogram. A polymorphism is indicated by bimodal frequency distribution curve with the antimode between the two populations. Antimode which separates the extensive metabolisers from poor metabolisers serves as a baseline to distinguish these two groups²⁰. A probit plot³⁹ or normal test variable (NTV) plot⁴⁰ also can be used to express the bimodal distribution.

Different probe drugs are used for *CYP2D6* phenotyping. Earlier phenotyping studies have been performed with debrisoquine and sparteine. Later dextromethorphan⁴¹, metoprolol⁴² and codeine⁴³ were also used for phenotyping *CYP2D6* activity.

The antimodes of this bimodal distribution in Caucasians are about 20, 0.3 and 12.6 for sparteine³, dextromethorphan^{3, 41} and debrisoquine^{3, 41}/metoprolol⁴² respectively. The metabolic ratio is a function of factors such as renal drug clearance as well as enzyme activity. Environmental factors may modify these variables, which may give rise to differences in the antimode of the MR between ethnic groups⁴⁴.

Dextromethorphan represents the only probe drug readily available as OTC drug in most of the countries¹¹. It is also considered safe for children and pregnant women³³. However metabolism of this drug proceeds simultaneously *via* other enzymes such as *CYP3A4* and results should therefore be interpreted with some caution¹¹. Blood⁵ and salivary^{45, 46} analysis also have been used for phenotyping studies.

Phenotyping has several drawbacks. It is hampered by a complicated protocol of testing, risks of adverse drug reactions, problem with incorrect phenotype assignment due to co-administration of drugs and confounding effect of disease³. This approach may be hampered in patients who concomitantly receive drugs that are metabolized by *CYP2D6* and/or inhibit this enzyme. As a consequence metabolite formation of the probe drug may be reduced despite a normal enzyme activity and the metabolic ratio in urine would indicate a poor metaboliser. Such apparent transformation of an EM-phenotype to a PM-phenotype is termed as phenocopying^{11, 47}.

However, phenotyping is the only approach to evaluate enzyme function. If post-translational variation contributes to the individual *CYP2D6* activity then phenotyping will be the only way to identify such phenomena¹¹. Phenotyping is useful in revealing drug-drug interactions or defect in overall process of drug metabolism³.

Genotyping

Genotyping involves identification of defined genetic mutation that give rise to the specific drug metabolism phenotype. These mutations include genetic alterations that lead to overexpression (gene amplification), absence of an active protein product (null allele), or production of a mutant protein with diminished catalytic capacity (inactivating allele)³.

DNA isolated from peripheral lymphocytes can be used for genotyping. Two commonly used methods in genotyping are PCR-RFLP method and allele-specific PCR³. In the former technique, specific region of the gene of interest is amplified by PCR followed by digestion of the amplified DNA product with restriction endonucleases. The size of the digestion products is easily evaluated by agarose gel electrophoresis with ethidium bromide staining and UV transillumination^{3, 48}.

Table 2. CYP2D6 alleles⁵

Allele	Changes	Xba 1 haplo-type(kb)	Trivial name	Effect	Enzyme activity	
					<i>In_vivo</i>	<i>In_vitro</i>
CYP2D6*1A	None	29	Wild-type		Normal	Normal
CYP2D6*1B	3828G>A	29			Normal (d,s)	
CYP2D6*1C	1978C>T		M4		Normal (s)	
CYP2D6*1D	2575C>A		M5			
CYP2D6*1E	1869T>C					
CYP2D6*1XN		42		N active genes Incr		
CYP2D6*2A	1661G>C; 2850C>T; 4180G>C	29	CYP2D6L	R296C; S486T	Decrease (dx, d)	Decrease
CYP2D6*2B	1039C>T; 1661G>C; 2850C>T; 4180G>C			R296C S486T		
CYP2D6*2C	1661G>C; 2470T>C; 2850C>T; 4180G>C					
CYP2D6*2D	2850C>T; 4180G>C		M10	R296C S486T		
CYP2D6*2E	997C>G; 1661G>C; 2850C>T; 4180G>C		M12	R296C S486T		
CYP2D6*2F	1661G>C; 1724C>T; 2850C>T; 4180G>C		M14	R296C S486T		
CYP2D6*2G	1661G>C; 2470T>C; 2575C>A; 2850C>T; 4180G>C		M16	R296C S486T		
CYP2D6*2H	1661G>C; 2480C>T; 2850C>T; 4180G>C		M17	R296C S486T		
CYP2D6*2J	1661G>C; 2850C>T; 2939G>A; 4180G>C		M18	R296C S486T		

Allele	Changes	Xba 1 haplo-type(kb)	Trivial name	Effect	Enzyme activity	
					<i>In_vivo</i>	<i>In_vitro</i>
<i>CYP2D6</i> *2K	1661G>C; 2850C>T; 4115C>T; 4180G>C		M21	R296C S486T;		
<i>CYP2D6</i> *2XN (N=2, 3, 4, 5 or 13)	1661G>C; 2850C>T; 4180G>C	42-175		R296C; S486T N active genes	Increase (d)	
<i>CYP2D6</i> *3A	2549A>del	29	<i>CYP2D6A</i>	Frameshift	None (d, s)	None (b)
<i>CYP2D6</i> *3B	1749A>G; 2549A>del			N166D; Framon; R296C; S486T	None (d, s)	
<i>CYP2D6</i> *4A	100C>T; 974C>A; 984A>G; 997C>G; 1661G>C; 1846G>A; 4180G>C	44, 29, 16+9	<i>CYP2D6B</i>	P34S; L91M; H94R; Splicing defect; S486T	None (d, s)	None (b)
<i>CYP2D6</i> *4B	100C>T; 974C>A; 984A>G; 997C>G; 1846G>A; 4180G>C	29	<i>CYP2D6B</i>	P34S; L91M; H94R; Splicing defect; S486T	None (d, s)	None (b)
<i>CYP2D6</i> *4C	100C>T; 1661G>C; 1846G>A; 3887T>C; 4180G>C	44/29	K29-1	P34S; Splicing defect; L421P; S486T	None	
<i>CYP2D6</i> *4D	100C>T; 1039C>T; 1661G>C; 1846G>A; 4180G>C			P34S; Splicing defect; S486T	None (dx)	
<i>CYP2D6</i> *4E	100C>T; 1661G>C; 1846G>A; 4180G>C			P34S; Splicing defect; S486T		
<i>CYP2D6</i> *4F	100C>T; 974C>A; 984A>G; 997C>G; 1661G>C; 1846G>A; 1858C>T; 4180G>C			P34S; L91M; H94R; Splicing defect; R173C; S486T		

Allele	Changes	Xba 1 haplo-type(kb)	Trivial name	Effect	Enzyme activity	
					<i>In_vivo</i>	<i>In_vitro</i>
CYP2D6*4G	100C>T; 974C>A; 984A>G; 997C>G; 1661G>C; 1846G>A; 2938C>T; 4180G>C			P34S; L91M; H94R; Splicing defect; P325L; S486T		
CYP2D6*4H	100C>T; 974C>A; 984A>G; 997C>G; 1661G>C; 1846G>A; 3877G>C; 4180G>C			P34S; L91M; H94R; Splicing defect; E418Q; S486T		
CYP2D6*4J	100C>T; 974C>A; 984A>G; 997C>G; 1661G>C; 1846G>A100 C>T;			P34S; L91M; H94R; Splicing defect;		
CYP2D6*4K	1661G>C; 1846G>A; 2850C>T; 4180G>C			P34S; Splicing defect; R296C; S486T	None	
CYP2D6*4X2		32+9			None	
CYP2D6*5	CYP2D6 deleted	11.5 or 13	CYP2D6D	CYP2D6 deleted	None (d, s)	
CYP2D6*6A	1707T>del	29	CYP2D6T	Frameshift	None (d, dx)	
CYP2D6*6B	1707T>del; 1976G>A	29		Frameshift G212E	None (s, d)	
CYP2D6*6C	1707T>del; 1976G>A; 4180G>C			Frameshift G212E S486T	None (s)	
CYP2D6*6D	1707T>del; 3288G>A			Frameshift G373S		
CYP2D6*7	2935A>C	29	CYP2D6E	H324P	None (s)	
CYP2D6*8	1661G>C; 1758G>T; 2850C>T; 4180G>C		CYP2D6G	Stop codon		

Allele	Changes	Xba 1 haplo-type(kb)	Trivial name	Effect	Enzyme activity	
					<i>In_vivo</i>	<i>In_vitro</i>
<i>CYP2D6</i> *9	2613- 2615delAGA	29	<i>CYP2D6C</i>	K281del	Decrease (b,s,d)	Decrease (b,s,d)
<i>CYP2D6</i> *10A	100C>T; 1661G>C; 4180G>C	44, 29	<i>CYP2D6J</i>	P34S; S486T	Decrease (s)	
<i>CYP2D6</i> *10B	100C>T; 1039C>T; 1661G>C; 4180G>C	44,29	<i>CYP2D6Ch 1</i>	P34S; S486T	Decrease (d)	Decrease (b)
<i>CYP2D6</i> *11	883G>C; 1661G>C; 2850C>T; 4180G>C	29	<i>CYP2D6F</i>	Splicing defect; R296C; S486T	None (s)	
<i>CYP2D6</i> *12	124G>A; 1661G>C; 2850C>T; 4180G>C	29		G42R; R296C; S486T	None (s)	
<i>CYP2D6</i> *13	<i>CYP2D7P</i> / <i>CYP2D6</i> hybrid. Exon 1 <i>CYP2D7</i> , exons 2-9 <i>CYP2D6</i>	29		Frameshift	None (dx)	
<i>CYP2D6</i> *14	100C>T; 1758G>A; 2850C>T; 4180G>C	29		P34S; G169R; R296C; S486T	None (d)	
<i>CYP2D6</i> *15	138insT	29		Frameshift	None (d, dx)	
<i>CYP2D6</i> *16	<i>CYP2D7P</i> / <i>CYP2D6</i> hybrid. Exons1-7 <i>CYP2D7P</i> -related, exons 8-9 <i>CYP2D6</i> .	11	<i>CYP2D6D2</i>	Frameshift	None (d)	
<i>CYP2D6</i> *17	1023C>T; 1638G>C; 2850C>T; 4180G>C	29	<i>CYP2D6Z</i>	T1071; R296C; S486T	Decrease (d)	Decrease (b)
<i>CYP2D6</i> *18	9 bp insertion in exon_9	29	<i>CYP2D6</i> (J9)		Decrease (s)	Decrease (b)
<i>CYP2D6</i> *19	1661G>C; 2539-2542 delAACT; 2850C>T; 4180G>C			Frameshift; R296C; S486T	None	

Allele	Changes	Xba 1 haplo-type(kb)	Trivial name	Effect	Enzyme activity	
					<i>In_vivo</i>	<i>In_vitro</i>
CYP2D6*20	1661G>C; 1973insG; 1978C>T; 1979T>C; 2850C>T; 4180G>C			Frameshift; L213P; R296C; S486T	None (m)	
CYP2D6*21	77G>A		M1	R26H		
CYP2D6*22	82C>T		M2	R28C		
CYP2D6*23	957C>T		M3	A85V		
CYP2D6*24	2853A>C		M6	1297L		
CYP2D6*25	3198C>G		M7	R343G		
CYP2D6*26	3277T>C		M8	1369T		
CYP2D6*27	3853G>A		M9	E410K		
CYP2D6*28	19G>A; 1661G>C; 1704C>G; 2850C>T; 4180G>C		M11	V7M; Q151E; R296C; S486T		
CYP2D6*29	1659G>A; 1661G>C; 2850C>T; 3183G>A; 4180G>C		M13	V136M; R296C; V338M; S486T		
CYP2D6*30	1661G>C; 1855-1863 9bp rep; 2850C>T; 4180G>C		M15	172-174FRP rep; R296C; S486T		
CYP2D6*31	1661G>C; 2850C>T; 4042G>A; 4180G>C		M20	R296C; R440H; S486T		
CYP2D6*32	1661G>C; 2850C>T; 3853G>A; 4180G>C		M19	R296C; E410K; S486T		
CYP2D6*33	2483G>T		CYP2D6*1C	A237S	Normal (s)	
CYP2D6*34	2850C>T		CYP2D6*1D	R296C		
CYP2D6*35	31G>A; 1661G>C; 2850C>T; 4180G>C		CYP2D6*2B	V111M; R296C; D486T	Normal (s)	

Allele	Changes	Xba 1 haplo-type(kb)	Trivial name	Effect	Enzyme activity	
					<i>In_vivo</i>	<i>In_vitro</i>
<i>CYP2D6</i> *35X2	31G>A; 1661G>C; 2850C>T; 4180G>C			V11M; R296C; S486T	Increase	
<i>CYP2D6</i> *36	100C>T; 1039C>T; 1661G>C; 4180G>C; gene conversion to <i>CYP2D7</i> in exon 9	44,29	<i>CYP2D6</i> Ch2	P34S; S486T	Decrease (d)	Decrease (b)
<i>CYP2D6</i> *37	100C>T; 1039C>T; 1661G>C; 1943G>A; 4180G>C;		<i>CYP2D6</i> *10D	P34S; S486T; R201H; S486T		
<i>CYP2D6</i> *38	2587- 2590delGACT		N2	Frameshift	None	
<i>CYP2D6</i> *39	1661G>C; 4180G>C			S486T		

b, bufuralol; d, debrisoquine; dx, dextromethorphan; s, sparteine

§ = Source: Homepage of the human cytochrome P450 (CYP) allele nomenclature committee.
Editors: Ingelman-Sundberg M, Daly AK and Nebert DW. (URL:<http://www.imm.ki.se/cypalleles>)

In allele specific PCR amplification, oligonucleotides specific for hybridizing with the common or variant alleles are used for parallel amplification reactions. Analysis for the presence or absence of the appropriate amplified product is accomplished by agarose gel electrophoresis^{49, 50}.

These genotyping methods require small amount of blood or tissue, are not affected by underlying diseases or drugs taken by the patient and provide results within 48-72 hours, allowing for rapid intervention³. The number of known defective alleles is growing and a total of more than 30 different defective *CYP2D6* and 55 *CYP2D6* variations have been identified¹⁸ (a current list of *CYP2D6* alleles are given in Table-2). However, it appears that depending on the ethnic group, genotyping for only 5-6 most com-

mon defective alleles will predict the *CYP2D6* phenotype with about 95-99% certainty^{18, 51}. For example, the most common *CYP2D6* variant alleles in the, Caucasian⁵², Chinese/Japanese⁵³ and Black African/Afro-American¹⁸ population are *CYP2D6**4, *10 and *17 respectively.

ETHNIC ASPECTS

Racial and ethnic studies of drug metabolism have shown substantial inter-population differences in the polymorphic distribution of *CYP2D6* activity and corresponding genetic materials. The prevalence of PM and UEM in different ethnic groups is shown in Table 3 and 4. This polymorphism has been extensively studied in Caucasians and Orientals with results consistently showing a prevalence of PMs of 5-10% in

Table 3. Prevalence of CYP2D6 poor metabolisers in different ethnic groups (phenotyping).

Ethnic group	probe	Total subjects	PM (%)	Reference
Caucasians				
Americans	dx	549	6.7	106
	dx	480	7.7	107
(Minnesota)	dx	280	6.1	108
Canadians	dx	223	6.7	87
	dx	210	7	109
	sp	83	7.2	110
	sp	48	8.3	111
British	db	258	8.9	10
	db	95	12.6	112
Australian	db	100	6.0	113
(Tasmanian)	db	152	8.6	114
French	dx	216	5.1	115
	dx	110	10	22
	dx	103	3.9	116
German	sp	360	5	9
	sp	205	3.4	117
	db	76	6.6	118
Italian	db	67	7.5	119
New Zealand	db	111	7.2	120
Switzerland	dx	268	9	41
Spanish	db	377	6.6	121
	db	258	5	122
Swedish	db	1011	6.82	123
	db	757	5.4	124
Danish	sp	349	9.2	125
	sp	301	7.3	126
Turks	db	326	3.4	127
	sp/db	106	3.8	128
Hungary	db	100	8.0	129
Uruguayan	dx	302	7.3	130
Finland (Finns)	db	211	5	35
	db	155	3.2	131
	db	107	5.6	132
(Lapps)	db	70	5.6	131
Greenlanders				
(Denmark)	sp	185	3.2	133
East	sp	300	3.3	134
West	sp	171	2.3	134
Amerindians (Panama)				
Ngawbe Guaymin	sp	97	5.2	135
	sp/db	84	5.9	136
Cuna	sp	210	-	137
	sp	51	-	138
Blacks				
American Blacks	dx	106	1.9	107
Nigerian	db/mb	137	-	139
	db	128	8.6	140
	db	123	8.3	52
African-American	dx	181	3.9	58
Zambian	db/mp	94/96	-	141
Zimbabwean	db	103	2	57
Tanzanian	sp	216	0.5	142
Ghanaian	db	80	6	143
South Africans	db	46	18.8	52

Ethnic group	probe	Total subjects	PM (%)	Reference
Ethiopians	db	115	1.8	61
Asians				
Chinese	db	695	1.01	123
	dx	175	0.6	144
	dx	98	1	53
	db	97	-	145
(Taiwan)	db	124	1.6	23
(Mainland)	mp	107	0.5	146
	mp	98	-	147
(Singapore)	db	96	3	148
Japanese	mp	295	-	146
	mp	292	0.3	149
	mp	262	1	150
	mp	200	0.5	147
	sp	84	2.3	151
Thai	db	173	1.2	44
Korean	mp	218	0.7	146
Sri Lanka (Sinhalese)	db	111	-	152
Jordanian	mp	65	1.5	59
Malay	db	97	2	145
Khmer (Cambodia)	db	98	2.1	153
Hmong (Minnesota)	dx	203	8.9	108
Egyptians	db	84	1.4	52
Saudi Arabians	db	92	0.1	154
Indonesian	mp	104	-	155
India				
Bombay	db	147	2	62
Chandigarh	dx	100	3	63
Kerala	dx	104	4.8	64
Karnataka	dx	100	4	65
Andhra Pradesh (Kakinada)	dx	111	1.8	65
(Hyderabad)	dx	146	3.2	66
Tamil Nadu	dx	139	3.6	156

db=debrisoquine, sp= sparteine, dx= dextromethorphan, mp= metoprolol, cd= codeine.

Caucasians (Europeans and white North Americans) and 1% in Orientals (Chinese, Japanese and Koreans). In these populations, there is a high correlation of metabolic ratios with different probe drugs for *CYP2D6*. The studies, which compared Oriental population with Caucasians, showed an interethnic difference in the metabolism of *CYP2D6* substrates⁵⁴⁻⁵⁶.

However, studies in African populations have yielded inconsistent results with prevalence of PMs ranging from 0-19%⁵⁷. There seems to be a regional variation among African population. The wide variation in the *CYP2D6* phenotype in black Africans suggest that the black population is not genetically homogeneous as is often assumed⁵⁷. Moreover, in some African

populations, there is a lack of metabolic co-segregation of different *CYP2D6* probe drugs^{57, 59}.

The ultra extensive metabolisers (UEM) are reported with a prevalence of 1.5-29% in different ethnic groups. The frequency of the *CYP2D6* gene duplication was found to be 2-3% among most European populations and a proportion of 12% in Turkish subjects. The carriers of gene duplication in Saudi Arabia⁶⁰ and Ethiopia⁶¹ are 21% and 29% respectively. The mechanism behind this high proportion of UEM awaits further elucidation.

In India, an earlier study using debrisoquine, among subjects resident in Bombay, reported 2% PM with

Table 4. Frequencies of poor metabolisers and the *CYP2D6* gene duplication in genotyped population.

Ethnic group	Total subjects	PM (%)	MxN (%)	Reference
White North Americans	464	5.8	2.2	51
Black North Americans	246	3.3	2.4	51
Germans	589	7.0	2.0	51
South Germans	195	7.7	1.5	51
North Spanish	147	5.4	5.1	51
South Spanish	217	2.8	3.5	51
Swedish	270	8.0	1.0	51
Spanish	258	5.0	1.0	122
French	265	8.4	1.9	51
Turks	404	1.5	5.8	51
Koreans	152	0.0	0.3	51
Chinese	113	0.0	1.3	51
Nicaraguans	137	3.6	1.1	51
Saudi Arabians	101	2.0	21	60
Ethiopians	122	1.8	29	61

PM = Poor metabolisers; MxN = Ultra extensive metabolisers (Gene duplication)

respect to *CYP2D6*⁶². A much more recent study with dextromethorphan showed a frequency of 3% PM in a North Indian population⁶³. In South India, subjects from Kerala⁶⁴, Karnataka⁶⁵, Andhra Pradesh⁶⁵ and Tamil Nadu¹⁵⁶ have been phenotyped in our laboratory using dextromethorphan as probe drug. In Kerala the PM frequency is 4.8%, Karnataka 4%, Andhra Pradesh 1.8% and in Tamil Nadu it is 3.6%. The average prevalence of PM in South India is 3.52% (with 95% confidence interval of 2.03-5.66%) which is higher than that reported with the Chinese (0-1%) population and lower than Caucasians (5-10%)⁶⁵. A similar study also has been reported with Hyderabad City population and the PM frequency observed was 3.2%⁶⁶.

DNA marker studies reported that Indian and European populations have a common Caucasoid ancestor and are genetically distinct from those of Oriental population⁶⁷. However, the studies of *CYP2D6* activity in India show that the Indian population is a separate group with the enzyme activity in between the Caucasian and Oriental subjects. The study of *CYP2C19* polymorphism in North Indian subjects (11%) also indicated that cytochrome P-450 activity

in Indian population is different from other ethnic groups⁶⁸. However very less information is available about the genetic analysis of *CYP2D6* gene in Indian population. Since UEM cannot be determined by only phenotyping, the prevalence of UEM in Indian population is not available.

CLINICAL SIGNIFICANCE

Polymorphic drug oxidation

If sparteine and debrisoquine were the only drugs affected by *CYP2D6*, the discovery of this polymorphism in drug oxidation would have been of theoretical interest because both these drugs cannot be regarded as essential drugs. However, further studies identified a variety of structurally different compounds, which are metabolized by the *CYP-2D6* enzyme. A current list is provided in Table 5.

Although *CYP2D6* is only a relatively minor form in human liver (1.5% of total cytochrome-P450 isoforms), it metabolizes upto one quarter of all prescribed drugs. This may be because many of the drugs metabolized by *CYP2D6* are targeted to the central nervous system⁶⁹.

Table 5. Substrates of CYP2D6.

Antihypertensives			
Alprenolol	Bufuralol	Bunitrolol	Bupranolol
Carteolol	Clonidine	Debrisoquine	Guanoxan
Indoramine	Losartan	Metoprolol	Nimodipine
Nitrendipine	Oxyphenolol	Propranolol	Timolol
Antiarrhythmics			
Amiodarone	Aprindine	Encainide	Flecainide
Mexiletine	Procainamide	N-propylajmaline	Propafenone
Sparteine			
Antidepressants			
Amiflavine	Amitriptyline	Brofaromine	Citalopram
Clomipramine	Desmethylcitalopram	Desipramine	Fluvoxamine
Fluoxetine	Imipramine	Maprotiline	Minaprine
Moclobemide	Nefazodone	Nortriptyline	Paroxetine
Tomoxetine	Tranlycypromine	Trimipramine	Venlafaxine
Neuroleptics			
Clozapine	Haloperidol	Levomepromazine	Olanzapine
Perphenazine	Pimozide	Risperidone	Sertindole
Thioridazine	Zuclopenthixol		
Opiates			
Codeine	Dihydrocodeine	Dextromethorphan	Ethylmorphine
Hydrocodone	Norcodeine	oxycodone	Tramadol
Chemotherapeutic agents			
Clotrimazole	Doxorubicin	Ketoconazole	Mefloquine
Pyrimethamine	Rifampicin	Ritonavir	Roxithromycin
Sulfasalazine			
Antihistamine			
Azelastine	Cinnarizine	Loratadine	Promethazine
Miscellaneous			
Apigenine	Budesonide	Chloral hydrate	Cyclobenzaprine
Dexfenfluramine	Dibucaine	Dihydroergotamine	Dolansetron
Ethinylloestradiol	Fenoterol	Formoterol	4-hydroxyamphetamine
Laudampsome	MDMA (ecstasy)	Methoxamine HCl	Methoxyamphetamine
Methoxyphenamine	Methoxypsoralen	Metoclopramide	MPTP
Nicergoline	Ondansetron	Perhexiline	Phenformin
Phenylpropanolamine	Quercitin	Serotonin	Tacrine
Tamoxifen	Tolterodine	Tropisetron	

Brosen and Grams suggest⁷⁰ that clinical significance of polymorphism can be evaluated by asking the following questions: Does the kinetics of an active principle of a drug depend significantly on a specific enzyme? Does the resulting pharmacokinetic variability have any clinical importance? Can the variation in response be assessed by direct clinical or paraclinical measurement? On the basis of these criteria, significance exists for those drugs for which plasma con-

centration measurement are considered useful and for which the elimination of the drug and/or its active metabolite is mainly determined by CYP2D6 enzyme⁷⁰.

The PM trait is characterized clinically by an impressive deficiency in forming the relevant metabolite(s) of affected substrate, which can result in either drug toxicity or inefficacy. The reverse in case of UEM³.

The polymorphism of *CYP2D6* is clinically more significant for tricyclic antidepressants, certain neuroleptics, antiarrhythmics, antihypertensives, β -blockers and morphine derivatives²⁵. For tricyclic antidepressants, both the PM and UEM phenotypes of *CYP2D6* are at risk of adverse reactions⁴⁷. PM individuals given standard doses of these drugs will develop toxic plasma concentrations, potentially leading to unpleasant side effects including dry mouth, hypotension, sedation and tremor or in some cases life threatening cardiotoxicity³.

For example, it has been reported that identical dosing regimen of imipramine in EM and PM patients showed the absolute concentrations of both the parent drug (imipramine) as well as its desmethyl metabolite (desipramine) are greater in PM individuals, resulting in reduced ratio of parent drug to metabolite in them⁷¹⁻⁷³. Here the N-methylation of imipramine to its pharmacologically active desmethyl metabolite desipramine is catalyzed primarily by CYP2D6, whereas the 2-hydroxylation of desipramine to its pharmacologically inactive metabolites is catalyzed by *CYP2D6*^{3, 71}.

Administration of *CYP2D6* substrates to UEM individual may result in therapeutic failure because plasma concentrations of active drug at standard doses will be far too low⁷⁴. The clinical presentation of UEM and PM patients are at times similar, leading to confusion in understanding the basis of adverse drug reaction. Because of lack of dose individualization, patients may be subjected to recurrent depressive episodes and may not respond to treatment. Patients requiring treatment with antidepressant or antipsychotic substrates of *CYP2D6* may begin the normal treatment regimen. Because of the long half-life of these drugs, toxic drug concentrations may take 5-7 weeks to develop. Therefore, it is suggested that the patients should be phenotyped before starting the treatment with drugs which are metabolized mainly by *CYP2D6* enzyme³. A recent US study showed that, in patients prescribed with psychiatric drugs that are *CYP2D6* substrates, adverse drug reactions were observed in every patient with inherited mutations inactivating the *CYP2D6* gene⁷⁵.

A lack of *CYP2D6* enzyme would be expected to result in reduced drug therapy effectiveness in instances where prodrugs requiring activation by

CYP2D6 are used. For example, the analgesic effect of tramadol is severely reduced in PMs⁷⁶. Similarly, following administration of the prodrug codeine, morphine could not be detected in the plasma of *CYP2D6* PMs⁷⁷⁻⁷⁹. On the contrary, severe abdominal pain, a typical adverse effect of morphine, was observed in all UEM treated with codeine¹⁸. Moreover, codeine produced prolongation of the orocaecal transit time only in EM subjects⁸⁰.

DRUG INTERACTIONS

Due to the high polymorphic character of *CYP2D6*, this enzyme is also the site of a number of drug interactions *in vivo*, which are of clinical significance. Substrates with a high affinity for the enzyme bind strongly to it and inhibit the metabolism of other compounds which have lower affinity. Consequently drug interaction occur in extensive as well as poor metabolisers⁴⁷. By using this knowledge, pharmacokinetic interactions can be anticipated as follows:

If drug A affects P450 enzyme X and if P450 enzyme X metabolises drugs B, C and D, then drug A should affect the metabolism of drug B, C and D.

This type of knowledge is also being used to decide which drugs to develop, because the inhibition of P450 enzyme is generally not the goal of treatment⁸¹.

The interaction of two substrates for *CYP2D6* can result in a number of clinical responses. The first pass metabolism of the substrate may be inhibited or the rate of elimination may be prolonged such that higher plasma concentration and associated pharmacodynamic responses may occur^{5, 47, 82-86}.

Inhibition of metabolism by *CYP2D6* can also lead to a lack of therapeutic response when the pharmacological action is dependent on the active metabolite^{3, 87}. Since *CYP2D6* is not inducible by enzyme inducing drugs, drug interactions due to enzyme induction are very unlikely to occur⁴⁷.

PATHOPHYSIOLOGICAL ASPECTS OF *CYP2D6* POLYMORPHISM

Involvement of *CYP2D6* and its variant alleles in the pathogenesis of certain diseases (either by activating xenobiotics or by involvement in neurotransmitter metabolism) is an interesting and yet unsettled area of research.

CYP2D6 polymorphism has been linked to susceptibility to various diseases including certain cancers, early onset of Parkinson's disease, systemic lupus erythematosus, pituitary adenomas, Balkon nephropathy and ankylosing spondylitis^{1,19,88-90}. Metabolic activation of a procarcinogen may proceed via *CYP2D6* which implies that a patient of extensive metaboliser phenotype forms higher amounts of the active compounds and therefore at a higher risk to develop cancer¹¹. The *CYP2D6* gene is responsible for the metabolism of known human carcinogens, including nitrosamines and, possibly, nicotine. In addition it is suggested that there may be endogenous substrates for *CYP2D6*, including tryptamine, a well-known neuroactive amine⁹⁰. However, the influence of *CYP2D6* allelic variance in different types of cancer is a controversy. When some studies suggested a role for *CYP2D6* in the development of cancer, several studies could not support this^{91,92}.

A variety of studies investigated a possible link of Parkinsonism to *CYP2D6* expression⁹³⁻⁹⁶. Other studies however, failed to show any relation of *CYP2D6* activity and Parkinsonism^{97,98}. These trials have been performed in different ethnic groups and as P450 gene structures show interethnic group differences, comparison of these experiments and extrapolation for one ethnic group to another appears to be rather questionable¹¹.

Thus determination of these genetic polymorphism may be of clinical value in predicting adverse or inadequate response to certain therapeutic agents and in predicting increased risk of environmental or occupational exposure-linked disease. The genotyping/phenotyping will lead to increased therapeutic efficacy, improved patient outcome and thus more cost-effective medication^{3,99,100}.

MOLECULAR GENETICS IN CLINICAL LABORATORY

In place of simple descriptive information provided by therapeutic drug monitoring, molecular genetics could produce information about why a patient may require a different dose, drug or treatment regimen before a therapy is instituted^{100,101}. It might also substantially reduce the need for hospitalization because of adverse drug reactions and its associated costs³.

Pharmacogenetic testing is currently used in only a limited number of teaching hospitals and specialist academic centers. It is well established in

Scandinavian countries. The most widely accepted application of pharmacogenetic testing is the use of *CYP2D6* genotyping to aid individual dose selection for drugs used to treat psychiatric illness. Several independent testing laboratories provide DNA based testing service for a range of pharmacogenetic polymorphisms to pharmaceutical industry and medical practice⁷⁵. However, in India, this system has not been developed.

The advantage of combining genotyping/phenotyping with therapeutic drug monitoring is that genotyping can predict the PM or UEM drug metabolism phenotypes, and this information can be used for dosage adjustment or selection of an alternative drug, which is not a substrate of *CYP2D6*. The cost/healthcare effectiveness of these paradigms has not been extensively studied. Although there would be considerable cost associated with screening all individuals before dosing with *CYP2D6* substrates of narrow therapeutic index, this cost may be offset by a reduction in costs associated with toxic episodes or therapeutic failure and subsequent intervention³.

Polypharmacy and over the counter drug purchase is very common in developing countries like India and Sri Lanka. Since *CYP2D6* is responsible for the metabolism of most of the commonly used drugs, this may result in severe drug interactions especially in the poor metabolisers. Routine phenotyping or genotyping may not be economical in developing countries. However, monitoring of *CYP2D6* enzyme activity is important for the patients who report adverse reactions with normal dose of the drugs. This may help the physicians in individualization of the therapy especially for long term drugs like anti-depressants and anti-hypertensive⁷⁰.

Since genotyping is more costly procedure than phenotyping and not commonly available in most of the hospitals, the latter is more preferred for routine analysis in developing countries. However, for patients undergoing concomitant therapy with the drugs, which can affect on *CYP2D6* activity, genotyping may be used.

IMPLICATION FOR DRUG DEVELOPMENT

The knowledge gained about these polymorphism studies should be incorporated into drug development at an early stage to determine whether or not the drug is metabolized by *CYP2D6* and hence

subject to genetic polymorphism. Since phase-1 clinical trials are carried out at a rather later time during drug development, usually five to seven years after the initial discovery, a strategy, which allows for an earlier recognition of this phenomenon would be desirable⁴⁷. Dosing regimens are normally established during the phase-1 evaluation of drugs and are based on studies of relatively small number of subjects. However, with respect to oxidation phenotype, this subjects may not be representative of the general population¹.

If it were possible to predict that the metabolism of a drug cosegregates with a known polymorphism at the preclinical stage, the decision on whether or not to pursue development of the drug would be facilitated⁴⁷. Several *in vitro* approaches have been developed which allow a prediction to be made during preclinical testing if the metabolism of a new drug is subject to genetic polymorphism^{102, 103}. Inhibitory monoclonal antibodies are available which determine cytochrome P450 substrate and product specificity^{104, 105}. It is obviously also prudent to exclude potentially susceptible individuals from phase-1 dose escalation trials. This can prevent PM healthy subjects or patients being exposed to additional risk of toxicity during phase-1 and 2 development¹.

There is currently great interest in the pharmaceutical industries in pharmacogenetics and an increasing number of companies are genotyping their clinical trial populations. Moreover, the knowledge of genetic variability in drug response is becoming an increasingly important component of the drug registration process⁶⁹.

CONCLUSION

Although the potential importance of genetic variability in drug response is generally acknowledged in academic circles, the pharmaceutical industry and the drug regulatory authorities, this is not yet the case in general practice and, indeed, in many clinical pharmacology departments. A greater awareness of this urgently needed. Since many of the drug metabolized by *CYP2D6* are CNS active agents with narrow therapeutic indices, drug over treatment and accumulation can give rise to symptoms similar to those of the disease itself. Doctors need to be aware of whether a drug they are prescribing is subject to pharmacogenetic variability and its importance and

potential drug interactions. Prescribing advice should highlight the possibility of drug interactions when multiple drugs are prescribed concomitantly.

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PROBIOTIC MILK MAY HELP PREVENT COMMON CHILDHOOD INFECTIONS

Probiotic milk (milk containing bacteria that colonise the intestine and stimulate antibody production) may slightly reduce respiratory infections among children attending day care centres, finds a study in BMJ. These findings suggest that these bacteria may help prevent common infections, particularly in high risk children.

Over a seven month winter period, 571 children attending day care centres in Helsinki, Finland received milk with or without the probiotic bacteria strain Lactobacillus GG. During the study, parents recorded any respiratory symptoms (fever, runny nose, sore throat, cough, chest wheezes, earache) gastrointestinal symptoms (diarrhoea, vomiting, stomach ache) and absences from the day care centre.

Although there were no significant differences between the groups in the number of days with respiratory or gastrointestinal symptoms, the actual number of days with symptoms was lower in the Lactobacillus group. Children in the Lactobacillus group also had fewer days of absence because of illness and required less antibiotic treatment.

Although encouraging, we do not yet have a final answer on whether probiotics are sufficiently effective in preventing common childhood diseases that they can be routinely recommended, writes Professor Christine Wanke of Tufts University School of Medicine in Boston, USA. However, she concludes: "the accumulating data suggest that these organisms may help prevent both respiratory and diarrhoeal diseases in children at increased risk of such infections, such as those in day care facilities or living in developing countries."

(Effect of long term consumption of probiotic milk on infections in children attending day care centres: double blind, randomised trial. <http://bmj.com/cgi/content/full/322/7298/1327>)