Molecular genetics of CYP2D6: Clinical relevance with focus on psychotropic drugs

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Cytochrome P450 CYP2D6 is the most extensively characterized polymorphic drug-metabolizing enzyme. A deficiency of the CYP2D6 enzyme is inherited as an autosomal recessive trait; these subjects (7% of Caucasians, about 1% of Orientals) are classified as poor metabolizers. Among the rest (extensive metabolizers), enzyme activity is highly variable, from extremely high in ultrarapid metabolizers, to markedly reduced in intermediate metabolizers. The *CYP2D6* gene is highly polymorphic, with more than 70 allelic variants described so far. Of these, more than 15 encode an inactive or no enzyme at all. Others encode enzyme with reduced, 'normal' or increased enzyme activity. The *CYP2D6* gene shows marked interethnic variability, with interpopulation differences in allele frequency and existence of 'population-specific' allelic variants, for instance among Orientals and Black Africans. The CYP2D6 enzyme catalyses the metabolism of a large number of clinically important drugs including antidepressants, neuroleptics, some antiarrhythmics, lipophilic β-adrenoceptor blockers and opioids. The present-day knowledge on the influence of the genetic variability in CYP2D6 on the clinical pharmacokinetics and therapeutic effects/adverse effects of psychotropic drugs is reviewed.

Keywords: antidepressants, CYP2D6, debrisoquine, neuroleptics, polymorphism

Introduction

Many drugs, especially lipophilic compounds such as psychotropics need to be metabolized prior to excretion in urine. Oxidative phase I catalysed metabolism by cytochrome P450 (CYP) enzymes plays a major role in this respect. In the 1960s it was shown that the 30- to 40-fold variability in plasma concentrations of the tricyclic antidepressant nortriptyline in patients treated with the same dose is due to a pronounced variation in the rate of metabolism of the drug [1, 2]. Twin studies further showed that the rate of metabolism had a strong genetic component [2] and in 1980 the 10-hydroxylation of nortriptyline was shown to be catalysed by the polymorphic debrisoquine/sparteine hydroxylase (CYP2D6) [3].

This short review deals with the molecular genetics of CYP2D6 and its clinical relevance. Many recent reviews and books related to various aspects of this are available for further reading [4–8].

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The CYP2D6 polymorphism

The discovery of the debrisoquine/sparteine hydroxylation polymorphism

In 1977, the hydroxylation of the antihypertensive drug debrisoquine was shown to be polymorphic in nature [9, 10]. Independently, Eichelbaum *et al.* [11] showed that the oxidation of sparteine was also polymorphic. The metabolic ratios (MR; parent drug/metabolite) of the two drugs were closely correlated [12], showing that the same enzyme, now termed CYP2D6, was responsible for the two metabolic reactions.

The incidence of poor metabolizers (PM) of debrisoquine/sparteine with deficient CYP2D6 activity has been investigated in many populations, in most of them with a fairly small number of subjects [13, 14]. Among 1011 Swedish Caucasians we found 69 (6.3%) PM of debrisoquine [15]. This incidence is very similar to that in other European Caucasian populations (7–10%)

[13, 14]. In collaboration with Lou and associates in Beijing it was shown that the incidence of PM among 695 Chinese was only 1.0% using the antimode established in Caucasian populations [15]. A similar low incidence of PM has been shown in Japanese [16] and Koreans [17].

Molecular genetics of the poor metabolizers

The gene encoding the CYP2D6 enzyme is localized on chromosome 22 [18]. Three major mutant alleles, now termed *CYP2D6*3, *4* and **5* [23] (Table 1), associated with the PM phenotype, were found early on in Caucasians [19–22]. In Swedish Caucasians, the *CYP2D6*4* allele occurs with a frequency of 22% and accounts for more than 75% of the mutant alleles in this population [23]. The *CYP2D6*4* allele is almost absent in Chinese and this is the reason for the low incidence (1%) of PM in this population compared with 6% in Caucasians [15]. The frequency of the gene deletion (*CYP2D6*5*) on the other hand is very similar, i.e. 4–6% in different populations (Table 1). The *CYP2D6* gene has turned out to be extremely polymorphic with more than 70 allelic variants described so far (http://www.imm.ki.se/CYPalleles/cyp2d6.htm). In addition to the *CYP2D6 *3*, **4* and **5*, alleles, a large number of low-frequency alleles associated with the PM phenotype have been identified. Usually a few variants account for most of the mutant alleles in a population. The alleles of importance may, however, vary between populations (see below), which needs to be taken into consideration when applying genotyping methodology in clinical research or patient care.

Alleles in Orientals and Africans encoding CYP2D6 with decreased activity

Our early studies comparing CYP2D6 activity between Swedish and Chinese subjects revealed that the distribution of the MR of Chinese extensive metabolizers (EM) was shifted to the right compared with Swedish EM ($P<0.01$) [15]. This showed that the mean rate of hydroxylation of debrisoquine was lower in Chinese EM compared with Caucasian EM. This right shift in MR in Orientals is due to the high frequency of a mutant *CYP2D6*10* allele [24, 25] with the SNP C188T causing a Pro34Ser amino acid substitution and an unstable enzyme with decreased catalytic activity [25] (Figure 1). The frequency of this *CYP2D6*10* allele is similar, about 50%, in Chinese, Japanese and Koreans, but extremely low among Caucasians (Table 1).

Masimirembwa *et al.* [27] found a right shift of the MR in black Zimbabweans, similar to that found in Orientals. A mutated allele encoding an enzyme with decreased activity was subsequently identified and named *CYP2D6*17*. The frequency of this allele was found to be 34% in Zimbabweans [27] (Table 1), 17% in Tanzanians [28], 28% in Ghanaians [29] and 9% in Ethiopians [30]. This and many other studies demonstrate the genetic heterogeneity of different black populations of Africa. There are thus three fairly population specific alleles with *CYP2D6*4* in Caucasians, **10* in Asians and **17* in Africans. These mutations must have occurred after the separation of the respective populations from each other.

In Caucasians and Orientals a close geno- and phenotype relationship has been demonstrated [23, 25, 26]. However, in studies in Ethiopia [30], Ghana [29] and Tanzania [28] a lower CYP2D6 activity in relation to genotype has been demonstrated, indicating that in Africa, environmental factors, e.g. infections or food constituents are probably of importance in addition to genetic factors.

Gene duplication, multiduplication and amplification as causes of increased CYP2D6 activity

The problems in treating PM of debrisoquine have been extensively discussed over the years since the discovery of the CYP2D6 polymorphism [14]. Much less attention has been given to patients at the other extreme, i.e. ultrarapid

Table 1 Frequency of *CYP2D6*1* or **2* alleles (causing 'normal' enzyme activity) and some alleles causing no or deficient CYP2D6 activity in three different ethnic populations.

CYP2D6 alleles	*Functional mutation*	*Consequence*	*Allele frequency (%)*		
			Swedish	*Chinese*	*Zimbabwean*
**1 or *2*			69	43	54
**3*	A2637 del	Frame shift	2	0	0
**4*	G1934A	Splicing defect	22	0–1	2
**5*	Gene deletion	No enzyme	4	6	4
**10*	C188T	Unstable enzyme	n.d.	51	6
**17*	C1111T	Reduced affinity	n.d.	n.d.	34

n.d. = not determined.
Data are from original publications [23, 25, 27] and reviews [4, 5].

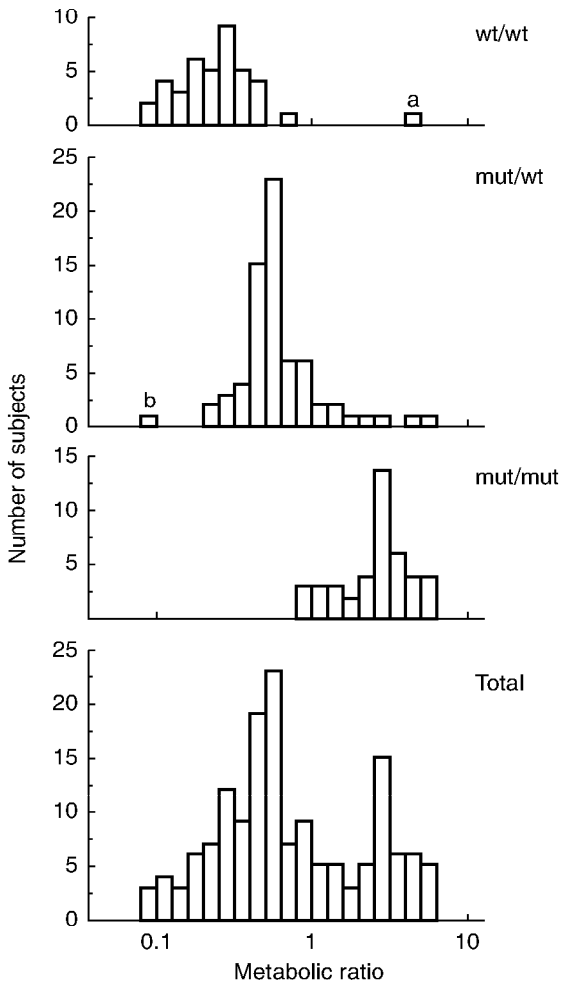

Figure 1 Distribution of the debrisoquine MR (parent drug/4-hydroxy metabolite) in three genotype groups related to the *CYP2D6*10* allele in 152 Korean subjects. wt = *CYP2D6*1*(or**2)* and mut = *CYP2D6*10*. Reproduced with permission from Roh *et al.* [26].

metabolizers. In 1993, we described a Swedish family with the father and his daughter and son having 12 extra copies of a functional *CYP2D6*2* gene in the *CYP2D* locus [31]. These subjects were ultrarapid metabolizers of debrisoquine with MR 0.01–0.02 (Figure 2). In another family, duplication of the *CYP2D6*2* gene was also associated with extremely high CYP2D6 activity [31]. This was the first demonstration of an inherited duplication/amplification of an active gene encoding a drug metabolizing enzyme. We also described two patients, who had to be treated with extremely high doses of antidepressants [32, 33]. One of the patients is further described below. The *CYP2D* locus of these patients was found to contain a duplication of the *CYP2D6*2* gene. A population study confirmed the association between the duplication/multiduplication of the *CYP2D6*2* gene and low debrisoquine MR [34].

In Swedish Caucasians the frequency of subjects having duplicated/multiduplicated genes is about 1–2% [34]. Going south in Europe, the reported frequency increases to 3.6% in Germany [35], 7–10% in Spain [36, 37] and 10% on Sicily in Italy [38]. The frequency is as high as 29% in black Ethiopians [30] and 20% in Saudi Arabians [39]. There is thus a European-African north–south gradient in the incidence of *CYP2D6* gene duplication. It has been speculated that the high incidence in Spain and Italy may have an ancestry in the Arabian conquest in the Mediterranean area [36]. Caucasian subjects with a *CYP2D6* gene duplication have been shown to be ultrarapid metabolizers of debrisoquine with MR usually between 0.01 and 0.15 [31, 34–36]. In the study of Aklillu *et al.* [30], black Ethiopians with multiple *CYP2D6* genes had higher MR, usually between 0.1 and 1. These subjects do thus not have the ultrarapid metabolism of debrisoquine demonstrated for Caucasians with multiple genes. This might be due to environmental factors in Africa causing a decreased activity.

Clinical relevance of the CYP2D6 polymorphism

Since the discovery of the CYP2D6 polymorphism almost 100 drugs have been shown to be substrates for this enzyme. Some of these drugs are shown in Table 2. The clinical importance of the polymorphism depends on a number of factors including whether the parent compound, metabolite(s) or both are metabolized or formed by CYP2D6; whether the parent compound, the metabolite(s) or both are active; the potency of the active species; and the overall contribution of the CYP2D6-dependent pathway to the clearance of the drug. Furthermore, the therapeutic index of the drug (narrow-broad), possible saturation of the CYP2D6-dependent pathway, and the contribution of other pathways of elimination need to be considered. Thus, the clinical impact of CYP2D6 dependent metabolism needs to be carefully investigated for each substrate. So far, the majority of *in vivo* data on the role of CYP2D6 are from single-dose pharmacokinetic studies. The increasing availability of genotyping methods has made clinical studies in patients receiving therapeutic doses possible. We will here highlight these aspects with some examples, mainly from the field of psychopharmacology.

Nortriptyline

Nortriptyline was one of the first clinically important drugs to be shown to be metabolized by CYP2D6 [3, 40]. These early studies (prior to the era of genotyping) were

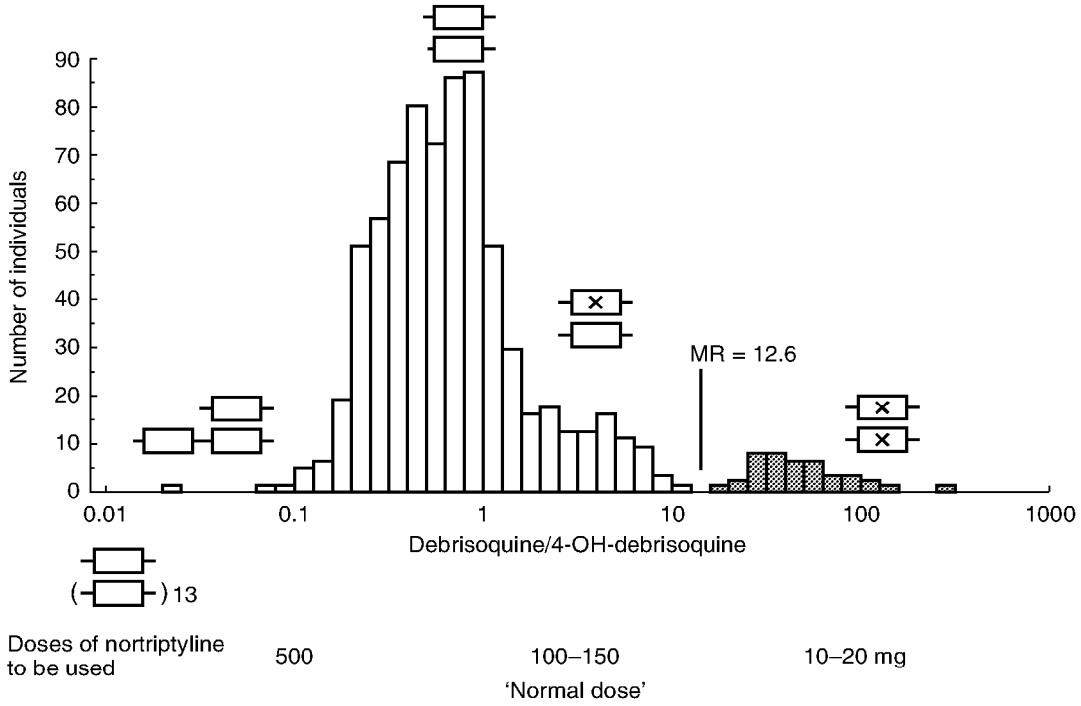

Figure 2 Distribution of the urinary debrisoquine MR in 757 healthy Swedish subjects with schematic presentation of *CYP2D6* genotypes, where a cross in an allele indicates a detrimental mutation. Also tentative doses of nortriptyline to be used in different genotypes are indicated. From [64].

Table 2 Some drugs whose metabolism is catalysed by CYP2D6.

β-Adrenoceptor blockers	*Antidepressants*	*Neuroleptics*
Metoprolol	Amitriptyline	Haloperidol
Propranolol	Clomipramine	Perphenazine
Timolol	Desipramine	Risperidone
	Fluoxetine	Thioridazine
Antiarrhythmic drugs	Fluvoxamine	Zuclopenthixol
Encainide	Imipramine	
Flecainide	Mianserin	*Miscellaneous*
Perhexilene	Nortriptyline	Codeine
Propafenone	Paroxetine	Debrisoquine
Sparteine	Venlafaxine	Dextromethorphan
		Phenformin
		Tolterodine
		Tramadol

performed in phenotyped panels of healthy subjects and the results have been confirmed *in vivo* in patients as well as *in vitro* using human liver microsomes and expressed enzymes. In a recent study by Dalén *et al.* [41], nortriptyline was given as a single oral dose to 21 healthy Swedish Caucasian subjects with different *CYP2D6* genotypes. As seen in Figure 3, there was a decrease in the plasma concentrations of nortriptyline from subjects with 0 functional genes (*CYP2D6*4/*4* genotype) to those with 1, 2 and 3 (gene duplication) functional genes. The plasma concentrations of the parent drug were extremely low in one subject with 13 *CYP2D6*2* genes. The plasma concentrations of the main metabolite 10-hydroxynortriptyline showed the opposite pattern, i.e. highest concentrations in the subject with 13 functional genes and lowest in the PM (Figure 3). This study clearly shows the impact of the detrimental *CYP2D6*4* allele as well as of the duplication/amplification of the *CYP2D6*2* gene on the metabolism of nortriptyline [41]. A relationship between the *CYP2D6* genotype and steady-state plasma concentrations of nortriptyline has also been demonstrated in Swedish depressed patients treated with nortriptyline [42].

We used the single dose results of Dalén *et al.* [41] (Figure 3) to simulate steady-state concentrations of nortriptyline in the different genotype groups after different daily doses of the drug assuming linear kinetics (Figure 4). A dose of 25 mg three times daily, which is usually recommended as a starting dose, resulted in concentrations near the upper limit of the recommended therapeutic interval (200–600 nM) in subjects with 0 (PM) and 1 (heterozygotes) functional CYP2D6 genes (Figure 4, upper curves). Subjects with 2 or more functional genes fall below 200 nM. At the usually recommended daily dose of nortriptyline 150 mg, subjects with 0 or 1 functional genes would attain levels above

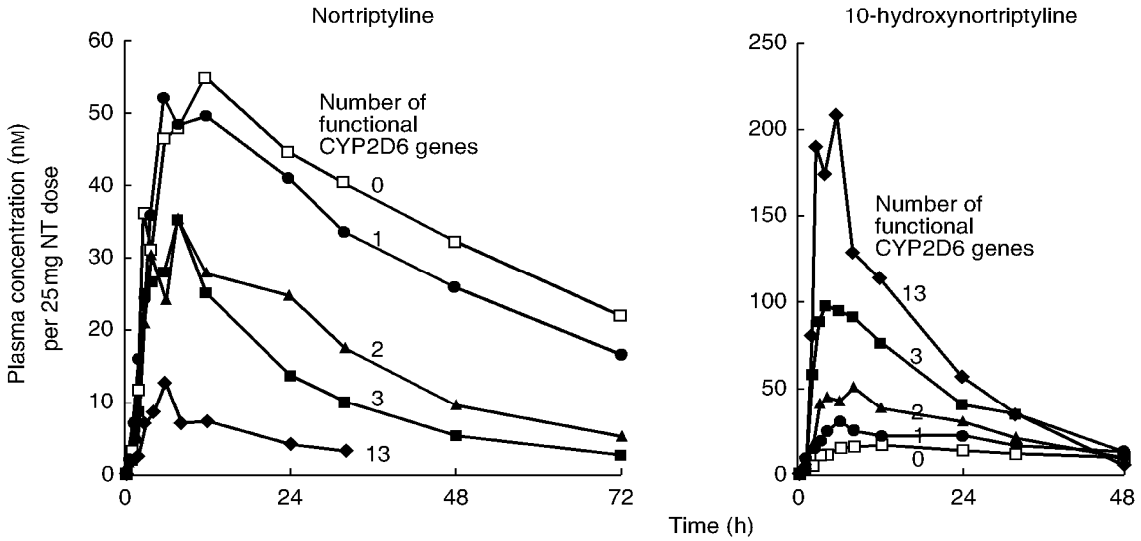

Figure 3 Mean plasma concentrations of nortriptyline (left) and 10-hydroxynortriptyline (right) in different *CYP2D6* genotype groups after a single oral dose of nortriptyline. The numerals close to the curves represent the number of functional *CYP2D6* genes in each genotype group. In groups with 0–3 functional genes, there were five subjects in each group while there was only one subject with 13 functional genes. Reproduced with permission from Dalén *et al.* [41].

600 nM and might therefore be at higher risk of developing adverse drug reactions. Subjects with 2 functional genes, who constitute about half of Caucasian populations, are in the middle of the therapeutic interval (Figure 4, middle curves). Subjects with gene duplication or amplification might require increased doses of nortriptyline, e.g. 75 mg three times daily (Figure 4, bottom curves) or higher. One out of 10 patients in Italy and Spain, where gene duplication is common, might require increased doses of CYP2D6 substrates. It should be underscored that the curves presented in Figure 4 are simulated from the single dose data of Figure 3 assuming linear kinetics. Early studies by Alexanderson [43] showed that single dose data of nortriptyline can be used to predict steady state concentrations. However, dose-dependent kinetics of this drug seem to occur in extensive metabolizers when high doses of nortriptyline saturate the capacity for hydroxylation [44, 45]. It should also be remembered that the 10-hydroxy metabolite of nortriptyline, formed by CYP2D6, is pharmacologically active although its relative contribution to the clinical effect and toxicity of nortriptyline has not been clearly elucidated [46]. The contribution of this metabolite is probably more important in ultrarapid metabolizers than in other patients.

Using the same protocol as in the study of Dalén *et al.* [41], we investigated the influence of the Oriental-specific *CYP2D6*10* allele on the disposition of nortriptyline in Chinese subjects living in Sweden [47]. Recently, Morita *et al.* [48] showed the influence of the *CYP2D6*10* allele on the steady-state plasma levels of nortriptyline and its 10-hydroxy metabolite in Japanese depressed patients. From these two studies it may be concluded that *CYP2D6*10* encodes an enzyme with decreased activity to metabolize nortriptyline. This effect is less pronounced than that of the Caucasian-specific *CYP2D6*4* allele, which encodes no enzyme at all.

Genotyping or phenotyping for *CYP2D6* may be a tool to predict proper initial dosing of drugs such as nortriptyline in individual patients, especially those with extremely low (PM) or high (UM) CYP2D6 activity. This can be demonstrated with our experience with two patients for whom the dosage of nortriptyline and other antidepressants needed to be individualized (Figure 2) [32, 33, 49, 50].

Patient 1. A 69 year old woman was hospitalized for moderate to severe depression and treated with nortriptyline in a modest dose of 25 mg three times daily. Two days after the start of treatment she complained of dizziness [49]. After a further 6 days of treatment, she complained of increasing tiredness and vertigo and appeared slightly confused. Low clearance of nortriptyline was suspected, blood was taken for nortriptyline analysis and the dosage was decreased to 25 mg once daily. The plasma concentration of nortriptyline after 8 days of treatment with the 75 mg daily dose was 1300 nM (recommended plasma concentration range 200–600 nM). The concentration on 25 mg daily for 12 days was 742 nM. When the dosage was further reduced to 20 mg at night, the patient had no side-effects and made an excellent recovery [49].

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