DRUG METABOLISM REVIEWS
Vol. 36, No. 2, pp. 243–277, 2004

Polymorphic Cytochrome P450 2D6: Humanized Mouse Model and Endogenous Substrates

Ai-Ming Yu,[1] Jeffrey R. Idle,[2] and Frank J. Gonzalez[1,*]

[1]Laboratory of Metabolism, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA
[2]Institute of Clinical Pharmacology, University of Bern, Bern, Switzerland

ABSTRACT

Cytochrome P450 2D6 (CYP2D6) is the first well-characterized polymorphic phase I drug-metabolizing enzyme, and more than 80 allelic variants have been identified for the *CYP2D6* gene, located on human chromosome 22q13.1. Human debrisoquine and sparteine metabolism is subdivided into two principal phenotypes—extensive metabolizer and poor metabolizer—that arise from variant *CYP2D6* genotypes. It has been estimated that CYP2D6 is involved in the metabolism and disposition of more than 20% of prescribed drugs, and most of them act in the central nervous system or on the heart. These drug substrates are characterized as organic bases containing one nitrogen atom with a distance about 5, 7, or 10 Å from the oxidation site. Aspartic acid 301 and glutamic acid 216 were determined as the key acidic residues for substrate-enzyme binding through electrostatic interactions. *CYP2D6* transgenic mice, generated using a lambda phage clone containing the complete wild-type *CYP2D6* gene, exhibits enhanced metabolism and disposition of debrisoquine. This transgenic mouse line and its wild-type control are models for human extensive metabolizers and poor metabolizers, respectively, and would have broad application in the study of *CYP2D6* polymorphism in drug discovery and development, and in clinical practice toward individualized drug therapy. Endogenous 5-methoxyindole-

*Correspondence: Dr. Frank J. Gonzalez, Laboratory of Metabolism, National Cancer Institute, National Institutes of Health, Building 37, Room 3106, 37 Convent Drive, Bethesda, MD 20892, USA; Fax: (301) 496-8419; E-mail: fjgonz@helix.nih.gov.

DOI: 10.1081/DMR-120034000
0360-2532 (Print); 1097-9883 (Online)

thylamines derived from 5-hydroxytryptamine were identified as high-affinity substrates of CYP2D6 that catalyzes their *O*-demethylations with high enzymatic capacity and specificity. Thus, polymorphic CYP2D6 may play an important role in the interconversions of these psychoactive tryptamines, including a crucial step in a serotonin-melatonin cycle.

Key Words: Cytochrome P450; CYP2D6; Polymorphism; Humanized mice; Drug metabolism; Pharmacokinetics; Debrisoquine; Dextromethorphan; Sparteine; Tryptamines; Beta-carbolines; Genotype; Phenotype; Parkinson's disease.

INTRODUCTION TO THE CYP2D6 POLYMORPHISM

Cytochrome P450 (P450 or CYP) enzymes, a superfamily of heme-thiolate proteins, are found in almost all living organisms and involved in the biotransformation of a diverse range of xenobiotics, including therapeutic drugs and countless toxins, and physiologically important hormones such as steroids, arachidonic acid, bile acids, and retinoic acid (Gonzalez and Nebert, 1990; Guengerich, 1997; Hasler, 1999; Ingelman-Sundberg et al., 1999; Nebert and Russell, 2002). Fifty-seven functional P450 genes have been identified in the human genome, among which only those encoding enzymes belonging to CYP1A, CYP1B, CYP2A, CYP2B, CYP2C, CYP2D, and CYP3A subfamilies contribute significantly to the biotransformation of exogenous chemicals. These P450s are mainly expressed in liver and to some extent in gut, kidney, and lung, and play a central role in drug metabolism and disposition. The efficacy of drug clearance is affected by many factors such as genetic variation (Bertilsson et al., 2002; Daly et al., 1996; Ingelman-Sundberg et al., 1999; Kroemer and Eichelbaum, 1995), transcriptional regulation (Akiyama and Gonzalez, 2003), and enzymatic inhibition and activation (Szklarz and Halpert, 1998; Tang and Stearns, 2001; Wienkers, 2001; Wrighton et al., 1996, 2000). In some cases, the metabolism of a drug results in its toxicity through bioactivation. Therefore, study of P450 enzymes has long been of interest for the prediction and identification of drug metabolism, drug–drug interactions and pharmacokinetic profile in drug discovery and development, and the prevention of adverse drug effects in clinical therapy (Daly, 1995; Evans and Relling, 1999; Guengerich, 1997; Nebert, 1997; Nebert and Russell, 2002).

Cytochrome P450 2D6 (CYP2D6) is one of the most important phase I drug-metabolizing enzymes, and it has been estimated to be involved in the oxidation of 20% to 30% drugs in clinical use, including many antiarrhythemics, antihypertensives, β-blockers, opioids, antipsychotics, and tricyclic antidepressants (Bertilsson et al., 2002; Evans and Relling, 1999; Ingelman-Sundberg et al., 1999; Kroemer and Eichelbaum, 1995; Nebert, 1997; Nebert and Russell, 2002).

CYP2D6 polymorphism was discovered independently in two laboratories in the late 1970s, due to the exaggerated responses to debrisoquine and sparteine in humans (Eichelbaum et al., 1979; Mahgoub et al., 1977), and thus commonly referred to as debrisoquine/sparteine polymorphism. Although debrisoquine predominantly undergoes 4-hydroxylation (Idle et al., 1979), sparteine was initially thought to be *N*-oxidized (Eichelbaum et al., 1979), then found to be metabolized through hydroxylation followed by dehydration (Ebner et al., 1995). Following these findings, a complete

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

cDNA encoding CYP2D6 protein was isolated in the late 1980s, and *CYP2D6* gene was traced to chromosome 22 (Gonzalez et al., 1987, 1988a,b; Kimura et al., 1989).

Poor metabolizer (PM) and extensive metabolizer (EM) are generally recognized as the two major CYP2D6 phenotypes (Eichelbaum, 1982; Evans et al., 1980; Schmid et al., 1985). As new information became available, the ultrarapid metabolizer (UM) and intermediate metabolizer (IM) subgroups were classified to yield a range of phenotypes with modestly decreased and increased activity, respectively (Bathum et al., 1998; Dahl et al., 1995; Daly, 1995; Raimundo et al., 2000). The incidence of CYP2D6 PM was investigated extensively in different ethnic populations containing small to large numbers of subjects. One study (Bertilsson et al., 1992) examined 1011 Swedish Caucasians and 695 Chinese and found that debrisoquine PMs occur among 6.28% of the Swedish Caucasian population and only 1.01% of the Chinese (Fig. 1). This finding is similar to results reported for European and American Caucasians (Alvan et al., 1990; Droll et al., 1998; Llerena et al., 1993; Marez et al., 1997; Nakamura et al., 1985; Sachse et al., 1997), and Japanese and Korean Orientals (Horai et al., 1989; Nakamura et al., 1985; Sohn et al., 1991) performed before and after that study. Moreover, debrisoquine hydroxylation in Asian EMs is slower than Caucasian EMs, as judged by the population mean of the metabolic ratio (MR; % dose excreted as debrisoquine/% dose excreted as 4-hydroxydebrisoquine). Most Caucasian EMs have an MR less than 1.0, whereas most Chinese EMs have an MR value of more than 1.0. As

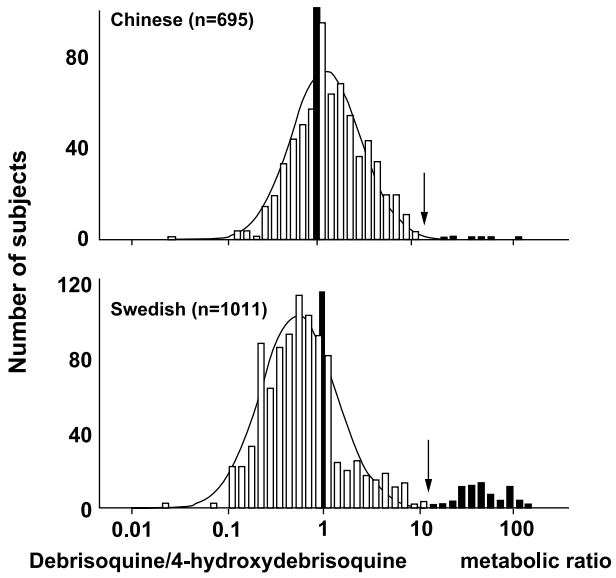

Figure 1. Shown is the distribution of urinary debrisoquine/4-hydroxydebrisoquine metabolic ratio (MR) in 695 Chinese and 1011 Swedish healthy subjects. The arrows indicate MR value of 12.6, an antimode between extensive metabolizers (EMs) and poor metabolizers. A line is drawn at MR = 1.0. Most Chinese EMs have MR > 1.0, whereas Caucasian EMs have MR < 1.0. This figure was reprinted from *Clinical Pharmacology & Therapeutics*, **51**(4), 1992. Pronounced differences between native Chinese and Swedish populations in the polymorphic hydroxylations of debrisoquin and S-mephenytoin, 388–397, (1992) with permission from Elsevier.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

shown in Fig. 1, urinary debrisoquine MR distribution is shifted to the right in Chinese EMs compared with Caucasian EMs.

More recently, the molecular basis of the *CYP2D6* polymorphism has been intensively studied. The *CYP2D6* gene exhibits more than 80 allelic variations among different ethnic populations (http://www.imm.ki.se/CYPalleles/cyp2d6.htm). The recessive PM phenotype occurs among individuals carrying two null *CYP2D6* alleles, arising from a broad range of DNA sequence variations, from single nucleotide substitution to deletion of the complete gene. This may result in a CYP2D6 protein that is unable to bind the substrate; a truncated protein unable to bind heme and, therefore, unable to produce recognizable P450 enzymatic activity; or simply no CYP2D6 protein at all (Haining and Yu, 2003). Other *CYP2D6* alleles contain point mutations resulting in one or more amino acid changes in the proteins compared with wild-type CYP2D6.1, and may lead to slightly decreased or increased activity (Yu et al., 2002). Generally, the *CYP2D6* polymorphism stratifies the population, depending on the copy number of wild-type alleles: PM, zero; IM, one; EM, two; and UM, multiple copies (Corchero et al., 2001; Gonzalez, 1996).

PHENOTYPE AND GENOTYPE

PMs lacking CYP2D6 activity are believed to be physiologically normal, although no comprehensive investigation has ever been carried out. However, the *CYP2D6* polymorphism is expected to influence the therapeutic efficacy and adverse drug reactions of common drugs such as β-blockers, selective serotonin reuptake inhibitors (SSRIs), and tricyclic antidepressants during clinical practice (Bertilsson et al., 2002; Gonzalez and Idle, 1994; Ingelman-Sundberg et al., 1999; Kroemer and Eichelbaum, 1995; Wolf and Smith, 1999; Wolf et al., 2000). For drug substrates with narrow therapeutic windows, serious consequences may result. Indeed, with fluoxetine (Prozac), a known substrate and inhibitor of CYP2D6, several phenotype-related fatality cases have been documented (Kincaid et al., 1990; Sallee et al., 2000). Nevertheless, it is not known whether these toxic events were related to drug metabolism. With the benefits of well-established phenotyping and rapidly developing genotyping methodologies, polymorphism information can be obtained and included in the patient's medical records. Here, it could be used to perform individualized drug therapy by adjusting the dose or selecting an alternative drug, which might reduce the incidence of similar adverse events (Bertilsson et al., 2002; Idle and Smith, 1995; Ingelman-Sundberg et al., 1999).

Over the years, several CYP2D6 phenotyping tests were developed, validated, and used in both genetic and clinical settings. Axiomatically, the best substrates for uncovering in vivo CYP2D6 polymorphism make capricious clinical tools and thus tend to fade into medical obscurity. The original debrisoquine (Evans et al., 1980; Mahgoub et al., 1977) and sparteine (Eichelbaum et al., 1979) phenotyping tests were gradually replaced by more clinically benign and durable tests, principally with dextromethorphan (Kupfer et al., 1984; Schmid et al., 1985), plus those involving metoprolol (Lennard et al., 1982a,b), bufuralol (Dayer et al., 1982), and codeine (Yue et al., 1989). The PM phenotype is assigned basically according to MRs greater than 0.3, 12.6, or 20, the antimode values for dextromethorphan/dextrorphan, debrisoquine/4-hydroxydebrisoquine, or sparteine/(2,3- plus 5,6-didehydrosparteine), respectively (Eichelbaum, 1982;

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

Evans et al., 1980; Schmid et al., 1985). In almost all respects, the rivalry between the different in vivo phenotyping tests was eclipsed by the development of DNA-based ex vivo genotyping tools (Daly et al., 1991; Gough et al., 1990; Heim and Meyer, 1990) that followed the cloning and characterization of the *CYP2D6* cDNA (Gonzalez et al., 1988a,b), and principal null alleles and the subsequent analysis of the organization *CYP2D* gene locus (Kimura et al., 1989).

Results obtained from phenotype–genotype correlation analysis are generally concordant with each other (Droll et al., 1998; Marez et al., 1997; Sachse et al., 1997), and have provided a genetic explanation for CYP2D6 polymorphism. One of these studies, including 672 unrelated European Caucasians (Marez et al., 1997), used dextromethorphan, debrisoquine, and sparteine as probe drugs for phenotyping, and polymerase chain reaction–single-strand conformation polymorphism (PCR–SSCP) analysis for genotyping. Among them, the frequency of the wild-type *CYP2D6*1A* allele (Kimura et al., 1989) is 32.2%. Major alleles (frequency) associated with the PM phenotype are *CYP2D6*4A* (Gough et al., 1990; Hanioka et al., 1990; Kagimoto et al., 1990) (15.6%), *CYP2D6*5* (Gaedigk et al., 1991; Steen et al., 1995) (6.9%), *CYP2D6*3* (Kagimoto et al., 1990) (1.6%), and *CYP2D6*6A* (Saxena et al., 1994) (0.8%). *CYP2D6*2* and *CYP2D6*2B* (Aklillu et al., 1996; Dahl et al., 1995; Johansson et al., 1993) alleles, associated with slightly reduced activity is present in 25.2% and 6.7% of this population, respectively. In addition, 29 novel mutations were identified by PCR–SSCP in this study (Marez et al., 1997).

The *CYP2D6*10* allele (Johansson et al., 1994; Yokota et al., 1993), containing the C188T, G1749C, and G4268C mutations, is found to be strongly associated with relatively lower CYP2D6 capacity in Asian populations at a high frequency of about 40% to 50% (Droll et al., 1998; Garcia-Barcelo et al., 2000; Tateishi et al., 1999; Teh et al., 2001). The *CYP2D6*17* allele (Masimirembwa et al., 1996), correlating with markedly decreased activity toward probe substrates, is common among African Americans and/or Africans at a frequency of 15% to 30% (Aklillu et al., 1996; Leathart et al., 1998; Wan et al., 2001; Wennerholm et al., 1999). *CYP2D6*9* (Broly and Meyer, 1993; Tyndale et al., 1991) occurs at relatively low frequencies (less than 4.0%) among these populations examined and encodes for the deletion of A2701–A2703 (Leathart et al., 1998; Teh et al., 2001; Tyndale et al., 1991). As expected, their modestly decreased catalytic activities are also observed with cDNA-transfected bacteria, yeast, insect, and mammalian cell membranes (Broly and Meyer, 1993; Fukuda et al., 2000; Johansson et al., 1994; Masimirembwa et al., 1996; Oscarson et al., 1997; Ramamoorthy et al., 2002; Tyndale et al., 1991; Yu et al., 2002), and genotyped and/or phenotyped human liver microsomes (Shimada et al., 2001; Zanger et al., 2001).

The UM phenotype, defined as subjects with debrisoquine MR less than 0.20 (Dahl et al., 1995) or sparteine MR less than 0.15 (Bathum et al., 1998), is reported to be present at relatively high frequency among Saudi Arabians (20%) (McLellan et al., 1997) and Ethiopians (29%) (Aklillu et al., 1996). This group of CYP2D6 phenotype can be explained by the occurrence of multiple copies of active *CYP2D6* alleles, and enhanced expression of stable and active protein among these populations (Aklillu et al., 1996; Dahl et al., 1995; Johansson et al., 1993). More recently, *CYP2D6*35* (Lovlie et al., 2001) was identified in Caucasian UMs without a CYP2D6 gene duplication (duplication negative) at significantly higher frequency than control EMs. However, in vitro functional analysis revealed that the enzymatic activity of its resulting allelic isoform CYP2D6.35 is comparable with the wild-type CYP2D6.1 isoform

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 1. Drug substrates and their metabolic pathways catalyzed by CYP2D6 and selected CYP2D6 inhibitors.

	Reaction	Reference
Psychotropic drugs		
Amitriptyline	Benzylic hydroxylation and *N*-demethylation	(Coutts et al., 1997; Ghahramani et al., 1997; Mellstrom et al., 1983; Olesen and Linnet, 1997b)
Citalopram	*N*-demethylation	(Rochat et al., 1997)
Clomipramine	Aromatic hydroxylation	(Balant-Gorgia et al., 1991)
Clozapine	*N*-demethylation	(Linnet and Olesen, 1997)
Imipramine	Aromatic hydroxylation	(Brosen et al., 1991; Su et al., 1993)
Desipramine	Aromatic hydroxylation	(Brosen and Gram, 1988; Su et al., 1993)
Fluoxetine	*N*-demethylation	(Hamelin et al., 1996)
Mianserine	Aromatic hydroxylation	(Koyama et al., 1996)
Mirtazapine	Aromatic hydroxylation	(Fawcett and Barkin, 1998)
Nortriptyline	Benzylic hydroxylation and *N*-demethylation	(Nordin et al., 1985; Olesen and Linnet, 1997a)
Paroxetine	*O*-demethylation	(Sindrup et al., 1992)
Venlafaxine	*O*-demethylation	(Ball et al., 1997)
Cardiovascular drugs		
Alprenolol	Aromatic hydroxylation	(Alvan et al., 1982)
Bufuralol	Aliphatic and aromatic hydroxylation	(Dayer et al., 1982, 1986; Mautz et al., 1995; Meyer et al., 1986)

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Encainide	*O*-demethylation	(Wang et al., 1984)
Flecainide	*O*-dealkylation	(Beckmann et al., 1988)
Metoprolol	Benzylic hydroxylation and *O*-demethylation	(Lennard et al., 1982a,b; Mautz et al., 1995)
Propafenone	Aromatic hydroxylation	(Botsch et al., 1993; Kroemer et al., 1991; Siddoway et al., 1987)
Propranolol	Aromatic hydroxylation	(Raghuram et al., 1984; Rowland et al., 1996; Yoshimoto et al., 1995)
Timolol	*O*-dealkylation	(Lewis et al., 1985)
Miscellaneous drugs		
Codeine	*O*-demethylation	(Dayer et al., 1988; Yue et al., 1989)
Debrisoquine	Aromatic and aliphatic hydroxylation	(Lightfoot et al., 2000; Mahgoub et al., 1977; Wolff et al., 1987)
Dextromethorphan	*O*- and *N*-demethylation	(Kupfer et al., 1984; Schmid et al., 1985; Yu et al., 2001)
Phenformin	Aromatic hydroxylation	(Oates et al., 1982)
Inhibitors		
Fluoxetine, Fluvoxamine, Norfluoxetine, Paroxetine, Quinidine, Sertraline		

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

(Allorge et al., 2001). Therefore, the role of *CYP2D6*35* allelic variant in duplication-negative Caucasian UMs requires further investigation.

Despite the evolution of PCR technologies since the 1990s, it still remains a challenge to forecast the CYP2D6 metabolic phenotype from a DNA-based genotyping assay both cheaply and rapidly. As the potential endogenous substrates were disclosed for CYP2D6, a third way of ''endogenous phenotyping'' was proposed (Yu et al., 2003b,c) that might obviate the problems inherent to both in vivo drug phenotyping and ex vivo DNA genotyping methods, and represents a new direction in need of study.

DRUG SUBSTRATES AND INHIBITORS

It has been estimated that CYP2D6 is responsible for 20% to 30% of the oxidation of prescribed drugs for humans (Bertilsson et al., 2002; Evans and Relling, 1999; Ingelman-Sundberg et al., 1999; Kroemer and Eichelbaum, 1995; Nebert, 1997; Nebert and Russell, 2002). Of particular note are the tricyclic antidepressants, SSRIs, 5-HT_3 receptor antagonists, antipsychotics, opiates, and amphetamines, together with the β-adrenoreceptor antagonists and the antidysrhythmic drugs, all agents that act either in the central nervous system (CNS) or on the heart (Table 1). CYP2D6 has also been shown to metabolize certain neurotoxins, including 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and its metabolite 1-methyl-4-phenylpyridine, which are believed to induce Parkinson's disease (PD) (Coleman et al., 1996; Fonne-Pfister et al., 1987; Gilham et al., 1997). A study revealed that CYP2D6 also contributes mainly to the metabolism of the psychotropic β-carboline alkaloids, harmaline and harmine (Yu et al., 2003d).

Various drugs of abuse are known as substrates (e.g., codeine, dextromethorphan, hydrocodone) or inhibitors [e.g., (-)-cocaine, pentazocine] of CYP2D6. Recreational drugs such as 3,4-methylenedioxymethamphetamine (''Ecstasy''), amphetamine, and methamphetamine are also oxidized by polymorphic CYP2D6. The metabolism and disposition, pharmacokinetics, and pharmacodynamics for some of these substrate drugs of abuse would be expected to vary among people due to CYP2D6 polymorphism. For other drugs, CYP2D6 may not contribute significantly to their overall disposition, but may catalyze the formation of highly active metabolites, such as codeine to morphine, hydrocodone to hydromorphone, and oxycodone to oxymorphone, and thus impact largely on their efficacy. In drug abuse, the CYP2D6 polymorphism is believed to play an important protective role as well as being a risk factor (Sellers and Tyndale, 2000; Sellers et al., 1997).

The best-known chemical inhibitor to CYP2D6 that is widely used in various studies is quinidine, with a inhibitory potency in the nanomolar range (Dayer et al., 1988, 1989; Hutzler et al., 2003; Otton et al., 1988; Yu and Haining, 2001a). By treatment with quinidine, CYP2D6 EMs can be converted to pseudo-PMs (i.e., phenocopies) (Ayesh et al., 1991). Interestingly, its stereoisomer, quinine, is a much less (about two orders of magnitude) potent inhibitor of CYP2D6 compared with quinidine. SSRIs display good inhibition to CYP2D6-catalyzed sparteine, dextromethorphan, and 5-methoxytryptamine oxidations with potency in the order of paroxetine > fluoxetine ≥ norfluoxetine > sertraline ≥ fluvoxamine > venlafaxine (Ereshefsky et al., 1995; Yu et al., 2003b). It is clear that the most potent CYP2D6

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

inhibitors belonging to SSRIs, fluoxetine and paroxetine, along with quinidine may cause serious drug–drug interactions in clinical practice (Ereshefsky et al., 1995; Kroemer and Eichelbaum, 1995).

These known CYP2D6 drug substrates and inhibitors are characterized as organic bases containing at least one nitrogen atom serving as an electron donor. The oxidation site, about 5 or 7 Å from the basic nitrogen, possesses a flat hydrophobic area close to it (de Groot et al., 1997; Koymans et al., 1992; Strobl et al., 1993). However, the distance between the basic nitrogen and reaction site is around 10 Å in a few of the substrates (de Groot et al., 1999a,b). Site-directed mutagenesis and molecular modeling revealed that the basic nitrogen atoms in the substrates can interact with the negatively charged carboxyl group of aspartic acid 301 and glutamic acid (de Groot et al., 1999a,b; Ellis et al., 1995; Guengerich et al., 2003; Paine et al., 2003). Thus, it is likely that both of these acidic amino acids are key residues for CYP2D6-substrate binding through electrostatic interactions. Besides, CYP2D6 may provide more than one binding orientation or site of metabolism for the same substrate (Yu et al., 2001, 2002).

Like other P450-catalyzed oxidations, most of the reactions mediated by CYP2D6 are aliphatic/aromatic hydroxylations and *O*-demethylation (Table 1). However, some drug (and other chemical) substrates are *N*-demethylated by CYP2D6, which was initially seen as an atypical and rare metabolic pathway, and is now a generally accepted pathway (Coutts et al., 1994; de Groot et al., 1999a) as more and more chemicals have been shown to undergo *N*-demethylation. Dextromethorphan, the widely used probe drug both in vitro and in vivo, was both *O*- and *N*-demethylated by highly purified and well-characterized CYP2D6 isoforms (Ramamoorthy et al., 2002; Yu and Haining, 2001a,b; Yu et al., 2001). A combined protein and pharmacophore model has also been generated for CYP2D6 in order to elucidate all these reactions including *N*-demethylation (de Groot et al., 1999a,b), which would provide helpful information for the research on drug metabolism and drug–drug interactions.

SUSCEPTIBILITY TO DISEASE

It is reasoned that the mutations and polymorphism of P450 genes might lead to altered individual risk of disease because these enzymes are responsible for the biosynthesis and biodegradation of physiological compounds, as well as the metabolism and disposition of environmental chemicals (Gonzalez and Idle, 1994; Guengerich, 2003; Huber et al., 2002; Ingelman-Sundberg, 2001). It is also known that few common diseases are monogenetic in origin; many diseases are caused by multiple factors such as multiple genes, diet, exposure to environmental factors, or a combination of these. Therefore, caution must be exercised before drawing a conclusion about the genetic determination of a certain disease.

More and more evidence has accumulated during the past decades, in support of the association of P450 genes with diseases (Guengerich, 2003; Huber et al., 2002; Ingelman-Sundberg, 2001). For examples, *CYP1B1* has been identified as a major genetic determinant of primary congenital glaucoma, besides the risk for developing prostate, ovarian, lung, and breast cancer. This has been confirmed by analysis of the *CYP1B1*-null mouse model (Libby et al., 2003). CYP19, also named aromatase, which produces estrogen from androgen, is associated with the risk of breast cancer

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

(Huber et al., 2002). Deficiency of *CYP27*, which encodes the mitochondrial sterol 27-hydroxylase playing a key role in bile acid biosynthesis, causes cerebrotendinous xanthomatosis, an autosomal recessive sterol storage disease characterized by the accumulation of a bile alcohol in diverse tissues. Almost all these associations can be bridged through a defect in biotransformation of endogenous compounds or activation of exogenous chemicals.

Numerous studies have been reported with the intention to link specific disease to polymorphic CYP2D6, for which exist large numbers of allelic variants with high frequencies, significant interethnic differences, and multiple drugs and chemical neurotoxin substrates. Those examined have included PD, Alzheimer's disease, and various types of cancer (Gonzalez and Idle, 1994). However, the results obtained from these association studies have been inconsistent, even with the determination of specific null alleles by genotyping. For the susceptibility to PD, *CYP2D6* has been the most extensively examined candidate gene, probably evoked by its metabolism of MPTP that causes immediate dopaminergic neuronal damage and irreversible Parkinsonism. MPTP is activated to neurotoxic MPP^+ by monoamine oxidase B, whereas it is detoxicated by *N*-demethylation, largely by CYP2D6. Thus, there have been many commentaries predicting a protective role for polymorphic CYP2D6 in MPTP-induced PD. The variable results of these studies on the association between *CYP2D6* genotype and PD may be attributed to many of the studies employing only small numbers of patients. Thus, a metaanalysis of 11 studies was carried out and showed a small, yet significant ($P = 0.01$) odds ratio (1.47) for the association between the poor metabolizer genotypes and PD (McCann et al., 1997). However, a study (Payami et al., 2001) containing 566 PD patients and 247 control subjects, using standard diagnostic and genotyping techniques, revealed that the *CYP2D6*4* allele, which is the most common variant among CYP2D6 PMs, is not associated with earlier PD onset. On the contrary, apolipoprotein E has been consistently identified to be associated with onset age of PD (Kruger et al., 1999; Maraganore et al., 2000; Zareparsi et al., 1997) and is so far the only recognized susceptibility gene. Although the causes of the common forms of PD are still unknown, it would be helpful to examine the major risk factors together, including candidate genes, age, family history, and environmental exposure markers. Chemicals such as β-carboline alkaloids contained in the diet or formed from its components are known for their neurotoxicity and induction of PD similarly to MPTP. CYP2D6 has been shown to be involved in their metabolism as well as CYP1A2 (Yu et al., 2003d).

HUMANIZED MOUSE MODEL FOR CYP2D6 POLYMORPHISM

Clinical studies are fundamental to the identification of human pharmacogenetic polymorphisms, and for the establishment of pharmacokinetic profiles and drug–drug interaction effects. However, to determine how a drug is metabolized, what toxic effects it might produce, or how pathophysiological conditions affect drug metabolism at early stages of drug development, animal models or in vitro systems must be developed. Due to marked differences between humans and experimental animals, the results from animal studies can be misleading and need to be interpreted cautiously. The *CYP2D* family in humans has a single active member *CYP2D6* that is highly

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

polymorphic, whereas rats and mice have at least five genes (Gonzalez and Nebert, 1990; Nelson et al., 1996). Debrisoquine is hydroxylated to 4-hydroxydebrisoquine by humans and by Sprague–Dawley rats. However, female Dark Agouti (DA) rats have been found to possess a low capacity to metabolize debrisoquine (Al-Dabbagh et al., 1981). Similarly, no significant formation of 4-hydroxydebrisoquine was detected by liver microsomes from three strains of mice and by purified CYP2D9-11 (Masubuchi et al., 1997). Although the female DA rat was proposed early on as a model for the human PM phenotype, in which to evaluate the role of the debrisoquine 4-hydroxylation polymorphism in drug and chemical toxicity (Al-Dabbagh et al., 1981). Employing two inbred strains of rat as models for two human phenotypes was soon recognized as having practical limitations. For example, using DA (PM) and Lewis (EM) female rats, it was proposed that the reduced hepatotoxicity of aflatoxin B_1 (AFB1) in the DA rat was due to its relative inability to activate metabolically AFB1 (Hietanen et al., 1986; Ritchie and Idle, 1982). Subsequently, it emerged that DA rats

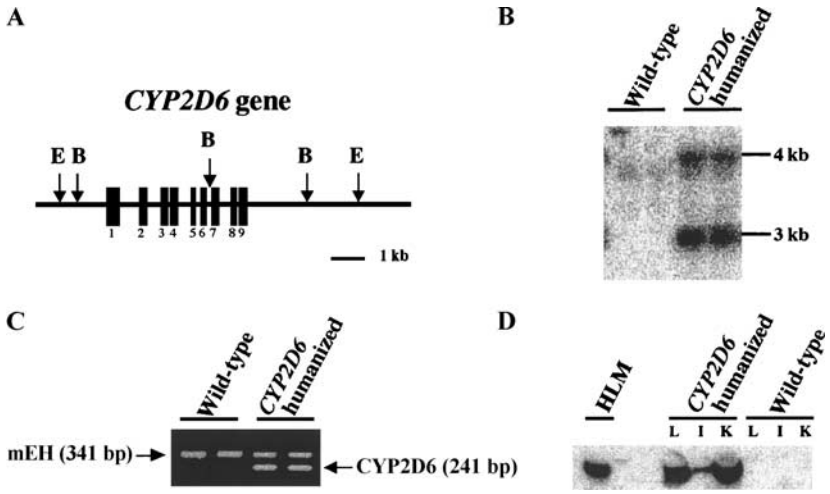

Figure 2. **A**, Generation and characterization of the *CYP2D6* transgenic (Tg-*CYP2D6*) mouse. Schematic diagram of the wild-type *CYP2D6* gene used for microinjection (Genbank accession number: M33388). Restriction sites for *Eco*RI (E) and *Bam*HI (B) are depicted. Black boxes represent *CYP2D6* exons. The bar represents 1 kb. **B**, Southern bolt genotyping of wild-type and Tg-*CYP2D6* mice. Tail DNA (15 μg) was digested with *Bam*HI and probed with *CYP2D6* cDNA. Hybridization signals were present only in Tg-*CYP2D6* mice, and their sizes were as expected from the *CYP2D6* sequence. **C**, PCR genotyping of wild-type and Tg-*CYP2D6* mice. Tail DNA was amplified with *mEH* [internal polymerase chain reaction (PCR) control] and *CYP2D6* gene-specific primers. The PCR products (341 bp for *mEH*, 241 bp for *CYP2D6*) were separated on a 1.5% agarose gel. **D**, Western blot analysis of *CYP2D6* protein expression in wild-type and Tg-*CYP2D6* mice. Liver (L), intestine (I), and kidney (K) microsomal proteins (40 μg) were separated by sodium dodecyl sulfate polyacrylamide gel electophoresis (SDSPAGE) and transferred to a nitrocellulose membrane. A CYP2D6-specific monoclonal antibody (Krausz et al., 1997) was used to assess CYP2D6 protein expression. The antibody only reacted against CYP2D6-expressed protein, but did not recognize any of the mouse CYP2D proteins. Human liver microsomes (HLM) was used as a control.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

had the highest microsomal epoxide hydrolase activity of 22 rat strains tested (Oesch et al., 1983), and this would appear to be the best explanation of the observed interstrain difference in AFB1 activation and hepatotoxicity, rapid metabolic clearance of the procarcinogenic AFB1 exo-8,9-epoxide. Thus, inbred strains, with their manifold genetic and biochemical differences, are imperfect models for the investigation of the biological consequences of human single polymorphisms.

To circumvent all these problems, a transgenic mouse line expressing CYP2D6 would offer a unique approach to answering fundamental questions about the specific role of CYP2D6 in drug metabolism and drug interactions. Such experiments would be performed in the context of the entire animal, and overcome many limitations inherent in in vitro experiments. To this end, the complete wild-type allele of the human *CYP2D6* gene (Fig. 2), including its regulatory sequence, was microinjected into a fertilized FVB/N mouse egg, and a *CYP2D6* transgenic (Tg-*CYP2D6*) mouse line has been produced (Corchero et al., 2001). Tg-*CYP2D6* mouse carries 5 ± 1 copies of *CYP2D6* transgene per haploid genome. Active CYP2D6 enzyme is expressed in liver, intestine, and kidney of Tg-*CYP2D6* mice (Fig. 2), which was confirmed with a specific monoclonal antibody (Krausz et al., 1997).

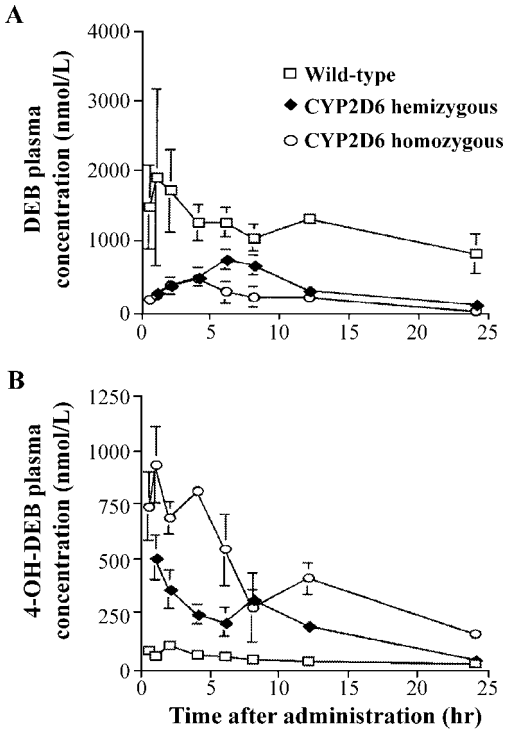

Figure 3. Time course of serum concentrations of debrisoquine (DEB) (**A**) and 4-hydroxydebrisoquine (4-OH-DEB) (**B**) from wild-type, *CYP2D6* transgenic heterozygous and homozygous mice after single oral administration of DEB (2.5 mg/kg). Venous blood was obtained 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 hr after DEB administration. Values represent the mean and the standard deviation (vertical lines) of DEB and 4-OH-DEB from 3 to 4 mice.

Metabolism and disposition of debrisoquine in Tg-*CYP2D6* mice is enhanced compared with control wild-type mice. After a single oral dose of debrisoquine (2.5 mg/kg), both Tg-*CYP2D6* heterozygous and homozygous mice had debrisoquine serum levels significantly lower than in wild-type (Fig. 3A). Consistently, 4-hydroxydebrisoquine levels are highest in Tg-*CYP2D6* homozygous, intermediate in Tg-*CYP2D6* heterozygous, and lowest in the wild-type (Fig. 3B). Pharmacokinetic analysis showed that the debrisoquine AUC is about three-fold and six-fold higher in wild-type mice than in heterozygous, and homozygous Tg-*CYP2D6* mice, respectively (Table 1). This is illustrated by differences in the elimination half-life of debrisoquine, which is 2.1 and 1.4 times shorter in the heterozygous and homozygous Tg-*CYP2D6* mice than in wild-type mice. Accordingly, Tg-*CYP2D6* mice showed a clearance about six- and three-fold higher than wild-type mice (Corchero et al., 2001).

CYP2D6 integration in the mouse genome does not affect any other physiological parameters such as renal function. Twenty-four hours after a single oral dose of debrisoquine, Tg-*CYP2D6* mice excreted significantly higher amounts of 4-hydroxydebrisoquine (28.9 ± 12.5% of dose) and lower amounts of debrisoquine (14.6 ± 6.4%) than the wild-type mice (6.2 ± 3.1% and 61.0 ± 9.0%, respectively). Urinary MR of debrisoquine for the wild-type mice was 12.1 ± 7.3%, which was decreased to 0.5 ± 0% with expression of the human transgene. Total recoveries of debrisoquine plus 4-hydroxydebrisoquine were 67.2 ± 10.7% and 43.5 ± 18.9% for the wild-type and Tg-*CYP2D6* mice, respectively (Corchero et al., 2001). This latter finding perhaps indicates that the human *CYP2D6* gene may provoke the metabolism of debrisoquine to other metabolites (Table 2).

Mutations of hepatocyte nuclear factor 4alpha (*HNF4α*) (Akiyama and Gonzalez, 2003; Hattersley, 1998; Ryffel, 2001), a hepatic transcription factor is known to regulate in vitro expression of the *CYP2D6* gene (Jover et al., 2001), could affect the disposition of CYP2D6 drug substrates. After deletion of *HNF4α* in Tg-*CYP2D6* mice,

Table 2. Pharmacokinetic parameters for debrisoquine and its metabolite, 4-hydroxydebrisoquine, after oral administration of 2.5 mg/kg of debrisoquine to wild-type, *CYP2D6* transgenic heterozygous and homozygous mice.[a]

	Wild-type	Heterozygote	Homozygote
Debrisoquine			
T_{max} (hr)	2.5 ± 1.8	6.7 ± 0.7	4.6 ± 1.8
C_{max} (nmol/L)	2940 ± 795	879 ± 128[b]	467 ± 61[b,c]
AUC_{0-24hr} (nmol.hr/L)	28400 ± 1840	8760 ± 1220[b]	4630 ± 1350[b,c]
CL (L/hr/kg)	15.2 ± 0.9	48.9 ± 6.4[b]	94.1 ± 22.3[b,c]
$T_{1/2}$ (hr)	16.5 ± 4.5	8.9 ± 2.1[b]	6.9 ± 1.6[b]
4-Hydroxydebrisoquine			
T_{max} (hr)	1.7 ± 0.3	3.3 ± 2.3	0.8 ± 1.2
C_{max} (nmol/L)	110 ± 12	535 ± 79[b]	1080 ± 97[b,c]
AUC_{0-24hr} (nmol.hr/L)	1090 ± 28	4630 ± 377[b]	9290 ± 931[b,c]

[a]Values represent the mean and the standard deviation from three to four mice.
[b]$P < 0.05$, values are significantly different from wild-type mice.
[c]$P < 0.05$, values are significantly different from heterozygous mice.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

debrisoquine 4-hydroxylation activity is significantly decreased more than 50%. With the Tg-*CYP2D6* mouse model, it is the first time that *CYP2D6* gene has been demonstrated to be regulated by *HNF4α* in vivo (Corchero et al., 2001).

The Tg-*CYP2D6* mouse model solves the problems of species differences, and offers a unique in vivo system to study drug metabolism and disposition, pharmacokinetics, and drug–drug interactions for the prediction of the effects of drugs, drug candidates, and environmental chemicals in humans. Moreover, this mouse line can serve as a whole intact animal model for exploring endogenous substrates for CYP2D6, investigating their biotransformations, and elucidating physiological significance and its polymorphism.

ENDOGENOUS SUBSTRATES FOR CYP2D6

Since the discovery of the *CYP2D6* polymorphism, there has been speculation about potential physiologically important substrates for CYP2D6 in humans (Kroemer and Eichelbaum, 1995; Llerena et al., 1989, 1993; Nadir et al., 1982). Could the PM have an advantage in development, reproduction, or behavior? The difference in personality between EM and PM individuals reported by Llerena and colleagues (Llerena et al., 1989, 1993) suggests that CYP2D6 may be involved in the metabolism of one or more endogenous neuroactive substances. This hypothesis is strongly supported by the expression of CYP2D6 in neurons of the human CNS, which has been demonstrated using a variety of techniques, including immunoblotting (Fonne-Pfister et al., 1987; Miksys et al., 2002; Siegle et al., 2001), in situ hybridization (Gilham et al., 1997; Siegle et al., 2001), reverse transcription-polymerase chain reaction (RT-PCR) (McFayden et al., 1998), and metabolism of the CYP2D6 probe drug dextromethorphan (Voirol et al., 2000) by microsomes prepared from brain tissues. One report localized the expression of CYP2D6 to the pigmented cells of the substantia nigra (Gilham et al., 1997), whereas another detected CYP2D6 mRNA in the neocortex, caudate nucleus, putamen, globus pallidus, hippocampus, thalamus, substantia nigra, and cerebellum (Siegle et al., 2001). CYP2D6 protein, however, was only detected in the large principal neurons in the cortex, hippocampus, and cerebellum (Siegle et al., 2001). If CYP2D6 was associated with the endothelial cells lining the 650 km of blood capillary found in the human brain, then a case could be made that it functioned as part of the blood–brain barrier and its role was as a ''last line of defense,'' preventing toxins from entering the brain, but this does not appear to be the case. Many toxic alkaloids, including MPTP-like β-carbolines, are CYP2D6 substrates (Yu et al., 2003d). However, all studies would appear to show that CYP2D6 within the CNS is neuronal in origin (Gilham et al., 1997; McFayden et al., 1998; Siegle et al., 2001), and this brings into question the function of this enzyme in the CNS. The possibility that CYP2D6 may have endogenous psychoactive substrates in the human brain would link all these evidence together and provide reasonable explanation for these phenomena.

Tryptamine, one of the trace amines found at very low concentrations in the mammalian CNS, but localized in neurons with a very high turnover and short half-life (Jones, 1982), exhibits high affinity to a new family of 15 G protein-coupled receptors recently identified (Borowsky et al., 2001). These receptors, called trace amine (TA) receptors, are distinct from the classical biogenic amine receptors, those for 5-HT,

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 3. Summary of apparent Michaelis-Menten constant (K_m) values for the biotransformations of probe drugs and biogenic amines catalyzed by recombinant CYP2D6.

Substrate	Reaction	Product	Expression system	K_m (μM)	Reference
Dextromethorphan	*O*-Demethylation	Dextrorphan	Yeast	1.7	(Fukuda et al., 2000)
			Yeast	1.3 ± 0.2	(Bapiro et al., 2002)
			Insect cells, purified	1.9 ± 0.2	(Yu et al., 2001)
Bufuralol	1′-Hydroxylation	1′-Hydroxybufuralol	Yeast	9.65 ± 0.3	(Bapiro et al., 2002)
			Insect cells	18.5 ± 7.3	(Evert et al., 1997)
Debrisoquine	4-Hydroxylation	4-Hydroxydebrisoquine	Yeast	55.2 ± 17	(Bapiro et al., 2002)
			Human cells	12.1 ± 1.3	(Granvil et al., 2002)
p-Tyramine	3-Hydroxylation	Dopamine	Yeast	190 ± 19.5	(Hiroi et al., 1998)
			Insect cells, purified	152 ± 52.3	(Haining and Yu, 2003)
			Bacteria, purified	1630 ± 80	(Miller et al., 2001)
m-Tyramine	4-Hydroxylation	Dopamine	Yeast	58.2 ± 13.8	(Hiroi et al., 1998)
			Insect cells, purified	75.1 ± 10.1	(Haining and Yu, 2003)
			Bacteria, purified	67 ± 7	(Miller et al., 2001)
3-Methoxyphenethylamine	*O*-Demethylation	3-Hydroxyphenethylamine	Bacteria, purified	160 ± 17	(Miller et al., 2001)
4-Methoxyphenethylamine	*O*-Demethylation	4-Hydroxyphenethylamine	Bacteria, purified	111 ± 5	(Miller et al., 2001)
5-Methoxytryptamine	*O*-Demethylation	5-Hydroxytryptamine	Insect cells	17.1 ± 3.96	(Yu et al., 2003b)
5-MDMT	*O*-Demethylation	Bufotenine	Insect cells	12.4 ± 1.01	(Yu et al., 2003c)
Pinoline	*O*-Demethylation	6-HO-THBC	Insect cells	1.77 ± 0.25	(Yu et al., 2003c)
Harmaline	*O*-Demethylation	Harmalol	Insect cells	1.41 ± 0.24	(Yu et al., 2003d)
Harmine	*O*-Demethylation	Harmol	Insect cells	7.42 ± 1.04	(Yu et al., 2003d)

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

dopamine, and norepinephrine. Therefore, tryptamine may now be considered a true candidate neurotransmitter or neuromodulator, although its physiological function is still the subject of speculation. It has been reported that CYP2D6 mediated the deamination of tryptamine (Martinez et al., 1997), which, prior to that, was understood to be an monoamine oxidase (MAO)-dependent pathway (Sullivan et al., 1986). However, a study (Yu et al., 2003a), using recombinant cDNA expressed P450 and MAO isozymes, together with a highly specific anti-CYP2D6 monoclonal antibody, demonstrated that CYP2D6 and other human P450s are not involved in the deamination of tryptamine. This reaction is essentially performed by MAO-A followed by aldehyde reductase. These results exclude the possibility that tryptamine is an endogenous substrate of CYP2D6.

Other in vitro studies have shown that CYP2D6 mediates the production of tyramine from 4-methoxyphenylethylamine (Miller et al., 2001), which is further hydroxylated by CYP2D6 to yield dopamine (Hiroi et al., 1998; Miller et al., 2001). These findings were additionally confirmed by other investigations (Haining and Yu, 2003). However, CYP2D6-mediated hydroxylation and *O*-demethylation of these catecholamines showed relatively high Michaelis–Menten constant (K_m) values, all of them are more than 55 μM (Table 3), and they are unlikely important endogenous substrates for CYP2D6. After screening various of phenylethylamines and indolethylamines, 5-methoxytryptamine (5-MT), 5-methoxy-*N,N*-dimethyltryptamine (5-MDMT), and pinoline (6-methoxy-1,2,3,4-tetrahydro-β-carboline) were found to bind with CYP2D6 and produce type I binding spectra (Fig. 4). Estimated dissociation constant (K_s) values were 20, 28, and 0.5 μM for 5-MT, 5-MDMT, and pinoline, respectively, indicating that they are high-affinity substrates for CYP2D6 (Yu et al., 2003b,c). Recombinant CYP2D6 catalyzes the *O*-demethylation of 5-MT, 5-MDMT, and pinoline with high turnover (Table 3), whereas other human P450 enzymes did not significantly carry out these reactions (Fig. 5). 5-Methoxytryptamine, 5-MDMT, and pinoline *O*-demethylation activities were about 20-, 11-, and 35-fold greater in liver microsomes from Tg-*CYP2D6* mice, respectively, than those in liver microsomes from control mice. Moreover, the increased activities were completely inhibited by an anti-CYP2D6 monoclonal antibody (Fig. 6). Therefore, polymorphic CYP2D6 was suggested as a highly specific, high-affinity, high-capacity 5-methoxyindolethylamine *O*-demethylase (Yu et al., 2003c).

5-Methoxytryptamine is an endogenous trace amine that belongs to the group of pineal methoxyindoles that includes melatonin (MEL) (Galzin et al., 1988; Raynaud and Pevet, 1991b). 5-MT is believed to be formed by the deacetylation of MEL by arylacylamidase (Beck and Jonsson, 1981; Rogawski et al., 1979), but may also be formed by the methylation of serotonin (5-HT) by hydroxyindole *O*-methyltransferase (Balemans et al., 1980). 5-Methoxytryptamine has been found in rat raphe nuclei, rat, golden hamster, sheep and human pineal, and hamster plasma (Beck and Bosin, 1979; Beck et al., 1981, 1982; Raynaud and Pevet, 1991a; van Benthem et al., 1985). 5-Methoxytryptamine has very poor affinity for MEL receptors (Sugden et al., 1997; Zawilska and Nowak, 1996). Its MEL-like actions, such as its inhibition of sexual maturation in male rats, may be due to its metabolism to MEL by arylalkylamine *N*-acetyltransferase (Lang et al., 1985). Conversely, 5-MT has a high affinity for most 5-HT receptor types, including 5-HT1A, 5-HT1B, 5-HT1C, 5-HT1D, 5-HT1F, 5-HT2, 5-HT2B, 5-HT4, 5-HT6, and 5-HT7 (Baxter et al., 1994; Bertrand et al., 2000;

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

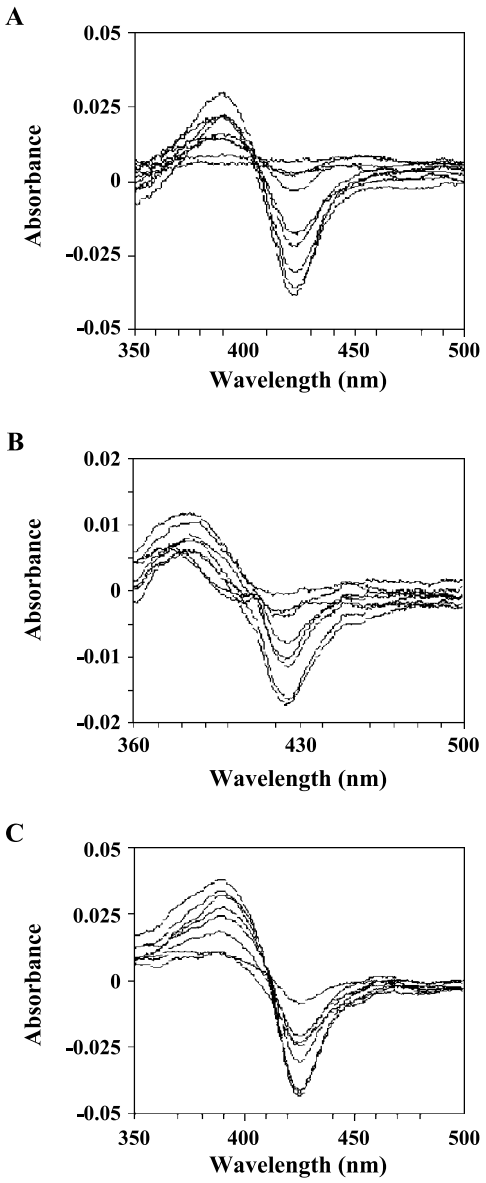

Figure 4. Binding spectra obtained with recombinant CYP2D6 and the sequentially added substrates (final concentration), 5-methoxytryptamine (1, 2, 5, 10, 20, 50, 100, 200, and 500 μM; **A**), 5-methoxy-*N*,*N*-dimethyltryptamine (5, 10, 20, 50, 100, 150, 200, and 300 μM; **B**), and pinoline (0.2, 0.5, 1, 2, 5, 10, 20, and 50 μM; **C**). The estimated K_s values were 20, 28, and 0.5 μM for 5-methoxytryptamine, 5-methoxy-*N*,*N*-dimethyltryptamine and pinoline, respectively.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

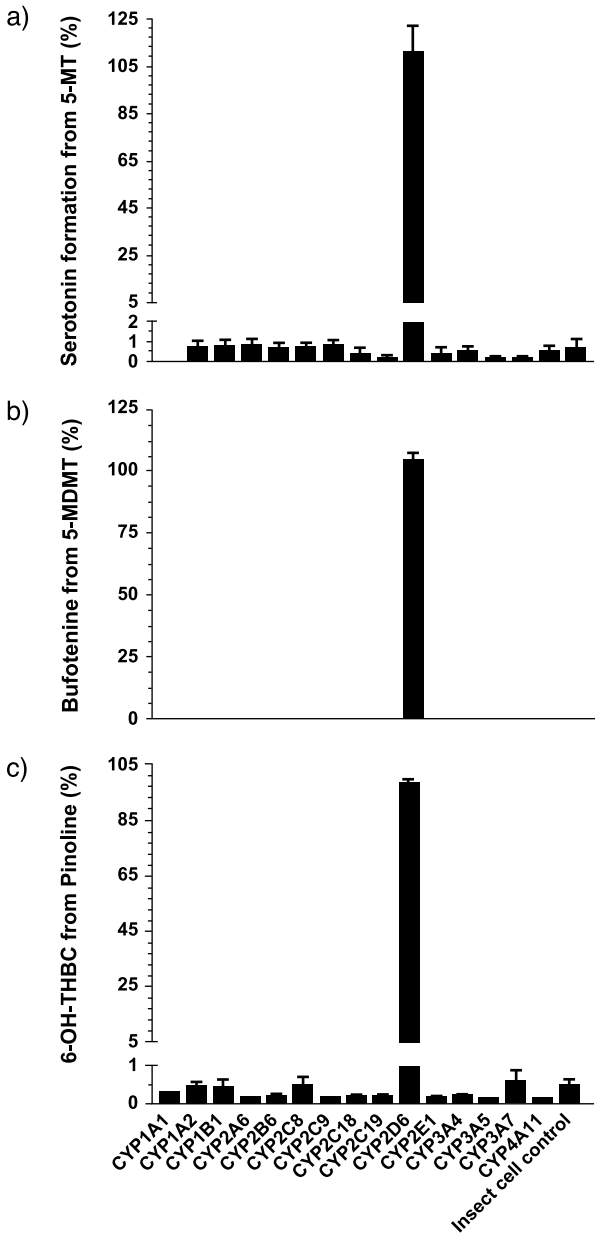

Figure 5. Specificity of CYP2D6 in the *O*-demethylations of 5-methoxytryptamine (5-MT) (a), 5-methoxy-*N*,*N*-dimethyltryptamine (5-MDMT) (b), and pinoline (c) using 15 recombinant common human P450 isozymes.

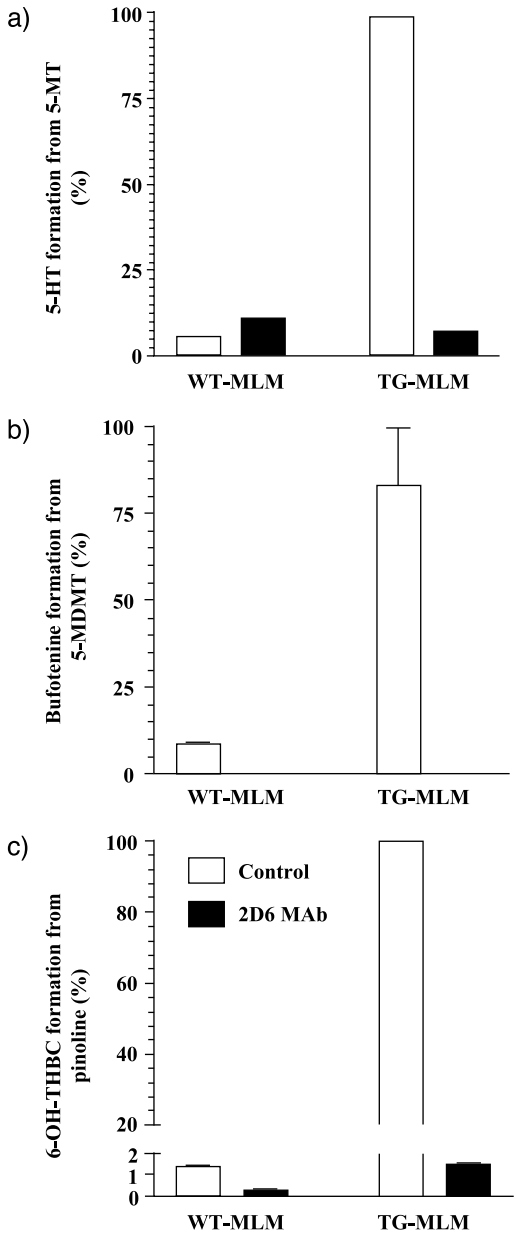

Figure 6. *O*-Demethylation of 5-methoxytryptamine (5-MT) (a), 5-methoxy-*N*,*N*-dimethyltryptamine (5-MDMT) (b), and pinoline (c) by mouse liver microsomes (MLM) from wild-type (WT) and *CYP2D6* transgenic (TG) mice, showing *O*-demethylase activity without (white bars, Control) and with (black bars, 2D6 MAb) the addition of anti-CYP2D6 monoclonal antibody.

Boess et al., 1997; Britt et al., 1988; Craig et al., 1990; Heuring and Peroutka, 1987; Hoyer et al., 1988; Peroutka, 1986; Schoeffter et al., 1988; Tsou et al., 1994; Wainscott et al., 1993). 5-Methoxytryptamine has been shown to possess little affinity for 5-HT3 receptors (Craig et al., 1990). In almost all cases where the receptor is coupled to a physiologic response, 5-MT is at least as potent an agonist at these 5-HT receptors as 5-HT. The regeneration of 5-HT from 5-MT catalyzed by CYP2D6 provides the missing link in the serotonin–melatonin cycle (Fig. 7).

5-Methoxy-*N*,*N*-dimethyltryptamine and 6-methoxy-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole, both of which are 5-HT derivatives like 5-methoxytryptamine (Fig. 7). These compounds share certain prominent chemical and biological similarities. Pinoline presents as normal constituents at remarkable high level (several μg/g) in pineal gland (Airaksinen and Kari, 1981a,b). 5-Methoxy-*N*,*N*-dimethyltryptamine, known as a potential endogenous ''psychotoxin,'' is biosynthesized in human pineal and detected in urine and pineal (Guchhait, 1976; Narasimhachari et al., 1971; van der Horst and Ebels, 1980). Meanwhile, these methoxyindolethylamines are present in retina at relatively high level (Leino and Airaksinen, 1985). Its CYP2D6-mediated metabolite bufotenine is also psychotropic with a pharmacology that resembles that of lysergic acid diethylamide, psilocin, and its parent molecule 5-MDMT, which is believed to involve 5-HT_{2A} and 5-HT_{2C} receptors (McBride, 2000; Ott, 2001). Their existence in the CNS is certain, but their biological roles are poorly understood. Whether the CYP2D6 polymorphism influences mood or behavior, or even neurological or psychiatric disease diathesis, via an interaction with one or more of this triad of endogenous CNS substrates, is merely speculation (Yu et al., 2003c).

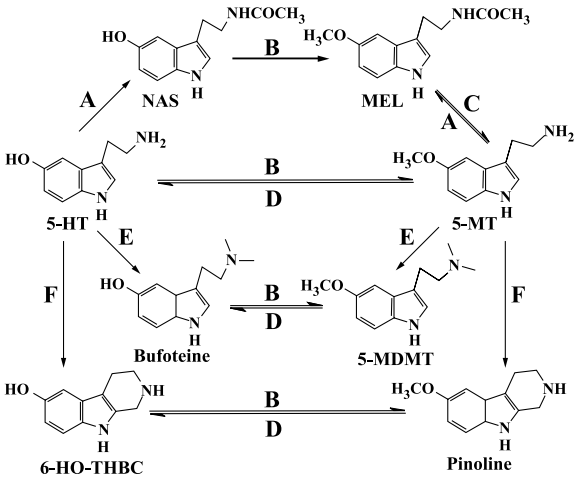

Figure 7. Interconversions of endogenous indolethylamines involving arylalkylamine *N*-acetyl transferase (AANAT, Reaction A), hydroxyindole *O*-methyltransferase (HIOMT, Reaction B), arylacylamidase (AAA, Reaction C), cytochrome P450 2D6 (CYP2D6, Reaction D), aromatic alkylamine *N*-methyltransferase (*S*-adenosylmethionine-dependent, Reaction E), and β-carboline formation, either spontaneously (Pictet–Spengler reaction) or from a N^5-methyltetrahydrofolate-dependent reaction (Reaction F).

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

The discovery of these physiological indolethylamine substrates of CYP2D6 may open the third way for the assignment of CYP2D6 phenotype. This ''endogenous phenotyping'' is different from the traditional phenotyping approach using a probe drug or forecasting from genotype determination, which is now common practice in both academic and industrial clinical pharmacology. It is also carried out in countless molecular epidemiological studies. There are limitations of precision, time, cost, and convenience that are associated with the various assay methodologies (Yu et al., 2003c). Thus, endogenous phenotyping may be of great value in a low-cost method for determining CYP2D6 and possibly other P450 polymorphism.

CONCLUSION

Since the discovery of debrisoquine/sparteine polymorphism in the late 1970s, significant interethnic difference in phenotype frequencies have been reported. Comprehensive studies on *CYP2D6* genotypes provided satisfactory molecular explanation for the distribution of phenotypes. Due to the lack of a robust animal model for the study of the CYP2D6 polymorphism, *CYP2D6* humanized mice have been generated and validated by molecular methods and debrisoquine phenotyping. This mouse model has been applied to the search for endogenous substrates for CYP2D6, which catalyzes the *O*-demethylation of a number of psychotropic methoxyindolethylamines. This mouse model could have broad applications for predicting the variation of metabolism and disposition of drugs or drug candidates, in vivo drug–drug interactions, and pharmacokinetics and pharmacodynamics for individualized drug therapy in the human population. This humanized mouse will also permit investigation into the physiological significance of these endogenous substrates of CYP2D6 and its polymorphism.

ACKNOWLEDGMENT

JRI wants to thank U.S. Smokeless Tobacco Co. for a grant for collaborative research.

REFERENCES

Airaksinen, M. M., Kari, I. (1981a). Beta-carbolines, psychoactive compounds in the mammalian body. Part I: occurrence, origin and metabolism. *Med. Biol.* 59(1):21–34.

Airaksinen, M. M., Kari, I. (1981b). Beta-carbolines, psychoactive compounds in the mammalian body. Part II: effects. *Med. Biol.* 59(4):190–211.

Akiyama, T. E., Gonzalez, F. J. (2003). Regulation of P450 genes by liver-enriched transcription factors and nuclear receptors. *Biochim. Biophys. Acta* 1619(3):223–234.

Aklillu, E., Persson, I., Bertilsson, L., Johansson, I., Rodrigues, F., Ingelman-Sundberg, M. (1996). Frequent distribution of ultrarapid metabolizers of debrisoquine in an

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Ethiopian population carrying duplicated and multiduplicated functional CYP2D6 alleles. *J. Pharmacol. Exp. Ther.* 278(1):441–446.

Al-Dabbagh, S. G., Idle, J. R., Smith, R. L. (1981). Animal modelling of human polymorphic drug oxidation–the metabolism of debrisoquine and phenacetin in rat inbred strains. *J. Pharm. Pharmacol.* 33(3):161–164.

Allorge, D., Harlow, J., Boulet, O., Hayhurst, G. P., Chowdry, J., Roth, E., Crewe, K., Lo-Guidice, J. M., Lhermitte, M., Broly, F., Tucker, G. T., Ellis, S. W. (2001). In-vitro analysis of the contribution of CYP2D6.35 to ultra-rapid metabolism. *Pharmacogenetics* 11(8):739–741.

Alvan, G., von Bahr, C., Seidemann, P., Sjoqvist, F. (1982). High plasma concentrations of beta-receptor blocking drugs and deficient debrisoquine hydroxylation. *Lancet* 1(8267):333.

Alvan, G., Bechtel, P., Iselius, L., Gundert-Remy, U. (1990). Hydroxylation polymorphisms of debrisoquine and mephenytoin in European populations. *Eur. J. Clin. Pharmacol.* 39(6):533–537.

Ayesh, R., Dawling, S., Hayler, A., Oates, N. S., Cholerton, S., Widdop, B., Idle, J. R., Smith, R. L. (1991). Comparative effects of the diastereoisomers, quinine and quinidine in producing phenocopy debrisoquine poor metabolisers (PMs) in healthy volunteers. *Chirality* 3(1):14–18.

Balant-Gorgia, A. E., Gex-Fabry, M., Balant, L. P. (1991). Clinical pharmacokinetics of clomipramine. *Clin. Pharmacokinet.* 20(6):447–462.

Balemans, M. G., Bary, F. A., Legerstee, W. C., Van Benthem, J. (1980). Seasonal variations in HIOMT activity during the night in the pineal gland of 21 day old male wistar rats. *J. Neural Transm.* 49(1–2):107–116.

Ball, S. E., Ahern, D., Scatina, J., Kao, J. (1997). Venlafaxine: in vitro inhibition of CYP2D6 dependent imipramine and desipramine metabolism, comparative studies with selected SSRIs, and effects on human hepatic CYP3A4, CYP2C9 and CYP1A2. *Br. J. Clin. Pharmacol.* 43(6):619–626.

Bapiro, T. E., Hasler, J. A., Ridderstrom, M., Masimirembwa, C. M. (2002). The molecular and enzyme kinetic basis for the diminished activity of the cytochrome P450 2D6.17 (CYP2D6.17) variant. Potential implications for CYP2D6 phenotyping studies and the clinical use of CYP2D6 substrate drugs in some African populations. *Biochem. Pharmacol.* 64(9):1387.

Bathum, L., Johansson, I., Ingelman-Sundberg, M., Horder, M., Brosen, K. (1998). Ultrarapid metabolism of sparteine: frequency of alleles with duplicated CYP2D6 genes in a Danish population as determined by restriction fragment length polymorphism and long polymerase chain reaction. *Pharmacogenetics* 8(2):119–123.

Baxter, G. S., Murphy, O. E., Blackburn, T. P. (1994). Further characterization of 5-hydroxytryptamine receptors (putative 5-HT2B) in rat stomach fundus longitudinal muscle. *Br. J. Pharmacol.* 112(1):323–331.

Beck, O., Bosin, T. R. (1979). Analysis of 5-methoxytryptamine in brain by gas chromatography mass spectrometry. *Biomed. Mass Spectrom.* 6(1):19–22.

Beck, O., Jonsson, G. (1981). In vivo formation of 5-methoxytryptamine from melatonin in rat. *J. Neurochem.* 36(6):2013–2018.

Beck, O., Jonsson, G., Lundman, A. (1981). 5-Methoxyindoles in pineal gland of cow, pig, sheep and rat. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 318(1):49–55.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

Beck, O., Borg, S., Lundman, A. (1982). Concentration of 5-methoxyindoles in the human pineal gland. *J. Neural Transm.* 54(1–2):111–116.

Beckmann, J., Hertrampf, R., Gundert-Remy, U., Mikus, G., Gross, A. S., Eichelbaum, M. (1988). Is there a genetic factor in flecainide toxicity? *BMJ* 297(6659):1316.

Bertilsson, L., Dahl, M. L., Dalen, P., Al-Shurbaji, A. (2002). Molecular genetics of CYP2D6: clinical relevance with focus on psychotropic drugs. *Br. J. Clin. Pharmacol.* 53(2):111–122.

Bertilsson, L., Lou, Y. Q., Du, Y. L., Liu, Y., Kuang, T. Y., Liao, X. M., Wang, K. Y., Reviriego, J., Iselius, L., Sjoqvist, F. (1992). Pronounced differences between native Chinese and Swedish populations in the polymorphic hydroxylations of debrisoquin and S-mephenytoin. *Clin. Pharmacol. Ther.* 51(4):388–397.

Bertrand, P. P., Kunze, W. A., Furness, J. B., Bornstein, J. C. (2000). The terminals of myenteric intrinsic primary afferent neurons of the guinea-pig ileum are excited by 5-hydroxytryptamine acting at 5-hydroxytryptamine-3 receptors. *Neuroscience* 101(2):459–469.

Boess, F. G., Monsma, F. J. Jr., Carolo, C., Meyer, V., Rudler, A., Zwingelstein, C., Sleight, A. J. (1997). Functional and radioligand binding characterization of rat 5-HT6 receptors stably expressed in HEK293 cells. *Neuropharmacology* 36(4–5):713–720.

Borowsky, B., Adham, N., Jones, K. A., Raddatz, R., Artymyshyn, R., Ogozalek, K. L., Durkin, M. M., Lakhlani, P. P., Bonini, J. A., Pathirana, S., Boyle, N., Pu, X., Kouranova, E., Lichtblau, H., Ochoa, F. Y., Branchek, T. A., Gerald, C. (2001). Trace amines: identification of a family of mammalian G protein-coupled receptors. *Proc. Natl. Acad. Sci. U. S. A.* 98(16):8966–8971.

Botsch, S., Gautier, J. C., Beaune, P., Eichelbaum, M., Kroemer, H. K. (1993). Identification and characterization of the cytochrome P450 enzymes involved in *N*-dealkylation of propafenone: molecular base for interaction potential and variable disposition of active metabolites. *Mol. Pharmacol.* 43(1):120–126.

Britt, S. G., Gonias, S. L., Sanders, J. M., Vandenberg, S. R. (1988). Agonist and antagonist activities of arylpiperazines at human platelet serotonin2 receptors. *J. Pharmacol. Exp. Ther.* 247(3):965–970.

Broly, F., Meyer, U. A. (1993). Debrisoquine oxidation polymorphism: phenotypic consequences of a 3-base-pair deletion in exon 5 of the CYP2D6 gene. *Pharmacogenetics* 3(3):123–130.

Brosen, K., Gram, L. F. (1988). First-pass metabolism of imipramine and desipramine: impact of the sparteine oxidation phenotype. *Clin. Pharmacol. Ther.* 43(4):400–406.

Brosen, K., Zeugin, T., Meyer, U. A. (1991). Role of P450IID6, the target of the sparteine-debrisoquin oxidation polymorphism, in the metabolism of imipramine. *Clin. Pharmacol. Ther.* 49(6):609–617.

Coleman, T., Ellis, S. W., Martin, I. J., Lennard, M. S., Tucker, G. T. (1996). 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is *N*-demethylated by cytochromes P450 2D6, 1A2 and 3A4—implications for susceptibility to Parkinson's disease. *J. Pharmacol. Exp. Ther.* 277(2):685–690.

Corchero, J., Granvil, C. P., Akiyama, T. E., Hayhurst, G. P., Pimprale, S., Feigenbaum, L., Idle, J. R., Gonzalez, F. J. (2001). The CYP2D6 humanized mouse: effect of the

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

human CYP2D6 transgene and HNF4alpha on the disposition of debrisoquine in the mouse. *Mol. Pharmacol.* 60(6):1260–1267.

Coutts, R. T., Su, P., Baker, G. B. (1994). Involvement of CYP2D6, CYP3A4, and other cytochrome P-450 isozymes in *N*-dealkylation reactions. *J. Pharmacol. Toxicol. Methods* 31(4):177–186.

Coutts, R. T., Bach, M. V., Baker, G. B. (1997). Metabolism of amitriptyline with CYP2D6 expressed in a human cell line. *Xenobiotica* 27(1):33–47.

Craig, D. A., Eglen, R. M., Walsh, L. K., Perkins, L. A., Whiting, R. L., Clarke, D. E. (1990). 5-Methoxytryptamine and 2-methyl-5-hydroxytryptamine-induced desensitization as a discriminative tool for the 5-HT3 and putative 5-HT4 receptors in guinea pig ileum. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 342(1):9–16.

Dahl, M. L., Johansson, I., Bertilsson, L., Ingelman-Sundberg, M., Sjoqvist, F. (1995). Ultrarapid hydroxylation of debrisoquine in a Swedish population. Analysis of the molecular genetic basis. *J. Pharmacol. Exp. Ther.* 274(1):516–520.

Daly, A. K. (1995). Molecular basis of polymorphic drug metabolism. *J. Mol. Med.* 73(11):539–553.

Daly, A. K., Armstrong, M., Monkman, S. C., Idle, M. E., Idle, J. R. (1991). Genetic and metabolic criteria for the assignment of debrisoquine 4-hydroxylation (cytochrome P4502D6) phenotypes. *Pharmacogenetics* 1(1):33–41.

Daly, A. K., Brockmoller, J., Broly, F., Eichelbaum, M., Evans, W. E., Gonzalez, F. J., Huang, J. D., Idle, J. R., Ingelman-Sundberg, M., Ishizaki, T., Jacqz-Aigrain, E., Meyer, U. A., Nebert, D. W., Steen, V. M., Wolf, C. R., Zanger, U. M. (1996). Nomenclature for human CYP2D6 alleles. *Pharmacogenetics* 6(3):193–201.

Dayer, P., Balant, L., Courvoisier, F., Kupfer, A., Kubli, A., Gorgia, A., Fabre, J. (1982). The genetic control of bufuralol metabolism in man. *Eur. J. Drug Metab. Pharmacokinet.* 7(1):73–77.

Dayer, P., Leemann, T., Kupfer, A., Kronbach, T., Meyer, U. A. (1986). Stereo- and regioselectivity of hepatic oxidation in man—effect of the debrisoquine/sparteine phenotype on bufuralol hydroxylation. *Eur. J. Clin. Pharmacol.* 31(3):313–318.

Dayer, P., Desmeules, J., Leemann, T., Striberni, R. (1988). Bioactivation of the narcotic drug codeine in human liver is mediated by the polymorphic monooxygenase catalyzing debrisoquine 4-hydroxylation (cytochrome P-450 dbl/bufI). *Biochem. Biophys. Res. Commun.* 152(1):411–416.

Dayer, P., Leemann, T., Striberni, R. (1989). Dextromethorphan *O*-demethylation in liver microsomes as a prototype reaction to monitor cytochrome P-450 db1 activity. *Clin. Pharmacol. Ther.* 45(1):34–40.

de Groot, M. J., Bijloo, G. J., Martens, B. J., van Acker, F. A., Vermeulen, N. P. (1997). A refined substrate model for human cytochrome P450 2D6. *Chem. Res. Toxicol.* 10(1):41–48.

de Groot, M. J., Ackland, M. J., Horne, V. A., Alex, A. A., Jones, B. C. (1999a). A novel approach to predicting P450 mediated drug metabolism. CYP2D6 catalyzed *N*-dealkylation reactions and qualitative metabolite predictions using a combined protein and pharmacophore model for CYP2D6. *J. Med. Chem.* 42(20):4062–4070.

de Groot, M. J., Ackland, M. J., Horne, V. A., Alex, A. A., Jones, B. C. (1999b). Novel approach to predicting P450-mediated drug metabolism: development of a combined protein and pharmacophore model for CYP2D6. *J. Med. Chem.* 42(9):1515–1524.

Droll, K., Bruce-Mensah, K., Otton, S. V., Gaedigk, A., Sellers, E. M., Tyndale, R. F. (1998). Comparison of three CYP2D6 probe substrates and genotype in Ghanaians, Chinese and Caucasians. *Pharmacogenetics* 8(4):325–333.

Ebner, T., Meese, C. O., Eichelbaum, M. (1995). Mechanism of cytochrome P450 2D6-catalyzed sparteine metabolism in humans. *Mol. Pharmacol.* 48(6):1078–1086.

Eichelbaum, M. (1982). Defective oxidation of drugs: pharmacokinetic and therapeutic implications. *Clin. Pharmacokinet.* 7(1):1–22.

Eichelbaum, M., Spannbrucker, N., Steincke, B., Dengler, H. J. (1979). Defective *N*-oxidation of sparteine in man: a new pharmacogenetic defect. *Eur. J. Clin. Pharmacol.* 16(3):183–187.

Ellis, S. W., Hayhurst, G. P., Smith, G., Lightfoot, T., Wong, M. M., Simula, A. P., Ackland, M. J., Sternberg, M. J., Lennard, M. S., Tucker, G. T., Wolf, C. R. (1995). Evidence that aspartic acid 301 is a critical substrate-contact residue in the active site of cytochrome P450 2D6. *J. Biol. Chem.* 270(49):29055–29058.

Ereshefsky, L., Riesenman, C., Lam, Y. W. (1995). Antidepressant drug interactions and the cytochrome P450 system. The role of cytochrome P450 2D6. *Clin. Pharmacokinet.* 29 Suppl 1:10–18. discussion 18–19.

Evans, W. E., Relling, M. V. (1999). Pharmacogenomics: translating functional genomics into rational therapeutics. *Science* 286(5439):487–491.

Evans, D. A., Mahgoub, A., Sloan, T. P., Idle, J. R., Smith, R. L. (1980). A family and population study of the genetic polymorphism of debrisoquine oxidation in a white British population. *J. Med. Genet.* 17(2):102–105.

Evert, B., Eichelbaum, M., Haubruck, H., Zanger, U. M. (1997). Functional properties of CYP2D6 1 (wild-type) and CYP2D6 7 (His324Pro) expressed by recombinant baculovirus in insect cells. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 355(3):309–318.

Fawcett, J., Barkin, R. L. (1998). Review of the results from clinical studies on the efficacy, safety and tolerability of mirtazapine for the treatment of patients with major depression. *J. Affect. Disord.* 51(3):267–285.

Fonne-Pfister, R., Bargetzi, M. J., Meyer, U. A. (1987). MPTP, the neurotoxin inducing Parkinson's disease, is a potent competitive inhibitor of human and rat cytochrome P450 isozymes (P450bufI, P450db1) catalyzing debrisoquine 4-hydroxylation. *Biochem. Biophys. Res. Commun.* 148(3):1144–1150.

Fukuda, T., Nishida, Y., Imaoka, S., Hiroi, T., Naohara, M., Funae, Y., Azuma, J. (2000). The decreased in vivo clearance of CYP2D6 substrates by CYP2D6*10 might be caused not only by the low-expression but also by low affinity of CYP2D6. *Arch. Biochem. Biophys.* 380(2):303–308.

Gaedigk, A., Blum, M., Gaedigk, R., Eichelbaum, M., Meyer, U. A. (1991). Deletion of the entire cytochrome P450 CYP2D6 gene as a cause of impaired drug metabolism in poor metabolizers of the debrisoquine/sparteine polymorphism. *Am. J. Hum. Genet.* 48(5):943–950.

Galzin, A. M., Eon, M. T., Esnaud, H., Lee, C. R., Pevet, P., Langer, S. Z. (1988). Day-night rhythm of 5-methoxytryptamine biosynthesis in the pineal gland of the golden hamster (*Mesocricetus auratus*). *J. Endocrinol.* 118(3):389–397.

Garcia-Barcelo, M., Chow, L. Y., Chiu, H. F., Wing, Y. K., Lee, D. T., Lam, K. L., Waye, M. M. (2000). Genetic analysis of the CYP2D6 locus in a Hong Kong Chinese population. *Clin. Chem.* 46(1):18–23.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Ghahramani, P., Ellis, S. W., Lennard, M. S., Ramsay, L. E., Tucker, G. T. (1997). Cytochromes P450 mediating the *N*-demethylation of amitriptyline. *Br. J. Clin. Pharmacol.* 43(2):137–144.

Gilham, D. E., Cairns, W., Paine, M. J., Modi, S., Poulsom, R., Roberts, G. C., Wolf, C. R. (1997). Metabolism of MPTP by cytochrome P4502D6 and the demonstration of 2D6 mRNA in human fetal and adult brain by in situ hybridization. *Xenobiotica* 27(1):111–125.

Gonzalez, F. J. (1996). The CYP2D subfamily. In: Ioannides, C., ed. *Cytochromes P450 Metabolic and Toxicological Aspects*. Boca Raton, FL: CRC Press, pp. 183–210.

Gonzalez, F. J., Idle, J. R. (1994). Pharmacogenetic phenotyping and genotyping. Present status and future potential. *Clin. Pharmacokinet.* 26(1):59–70.

Gonzalez, F. J., Nebert, D. W. (1990). Evolution of the P450 gene superfamily: animal–plant 'warfare,' molecular drive and human genetic differences in drug oxidation. *Trends Genet.* 6(6):182–186.

Gonzalez, F. J., Matsunaga, T., Nagata, K., Meyer, U. A., Nebert, D. W., Pastewka, J., Kozak, C. A., Gillette, J., Gelboin, H. V., Hardwick, J. P. (1987). Debrisoquine 4-hydroxylase: characterization of a new P450 gene subfamily, regulation, chromosomal mapping, and molecular analysis of the DA rat polymorphism. *DNA* 6(2):149–161.

Gonzalez, F. J., Skoda, R. C., Kimura, S., Umeno, M., Zanger, U. M., Nebert, D. W., Gelboin, H. V., Hardwick, J. P., Meyer, U. A. (1988a). Characterization of the common genetic defect in humans deficient in debrisoquine metabolism. *Nature* 331(6155):442–446.

Gonzalez, F. J., Vilbois, F., Hardwick, J. P., McBride, O. W., Nebert, D. W., Gelboin, H. V., Meyer, U. A. (1988b). Human debrisoquine 4-hydroxylase (P450IID1): cDNA and deduced amino acid sequence and assignment of the CYP2D locus to chromosome 22. *Genomics* 2(2):174–179.

Gough, A. C., Miles, J. S., Spurr, N. K., Moss, J. E., Gaedigk, A., Eichelbaum, M., Wolf, C. R. (1990). Identification of the primary gene defect at the cytochrome P450 CYP2D locus. *Nature* 347(6295):773–776.

Granvil, C. P., Krausz, K. W., Gelboin, H. V., Idle, J. R., Gonzalez, F. J. (2002). 4-Hydroxylation of debrisoquine by human CYP1A1 and its inhibition by quinidine and quinine. *J. Pharmacol. Exp. Ther.* 301(3):1025–1032.

Guchhait, R. B. (1976). Biogenesis of 5-methoxy-*N*,*N*-dimethyltryptamine in human pineal gland. *J. Neurochem.* 26(1):187–190.

Guengerich, F. P. (1997). Role of cytochrome P450 enzymes in drug–drug interactions. *Adv. Pharmacol.* 43:7–35.

Guengerich, F. P. (2003). Cytochrome P450s, drugs, and diseases. *Mol. Interv.* 3(4):8–18.

Guengerich, F. P., Hanna, I. H., Martin, M. V., Gillam, E. M. (2003). Role of glutamic acid 216 in cytochrome P450 2D6 substrate binding and catalysis. *Biochemistry* 42(5):1245–1253.

Haining, R. L., Yu, A. M. (2003). Cytochrome P450 pharmacogenetics. In: Lee, J., Obach, R. S., Fisher, M. B., eds. Drug Metabolizing Enzymes. FontisMedia, pp. 375–419.

Hamelin, B. A., Turgeon, J., Vallee, F., Belanger, P. M., Paquet, F., LeBel, M. (1996). The disposition of fluoxetine but not sertraline is altered in poor metabolizers of debrisoquin. *Clin. Pharmacol. Ther.* 60(5):512–521.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

Hanioka, N., Kimura, S., Meyer, U. A., Gonzalez, F. J. (1990). The human CYP2D locus associated with a common genetic defect in drug oxidation: a G1934—a base change in intron 3 of a mutant CYP2D6 allele results in an aberrant 3′ splice recognition site. *Am. J. Hum. Genet.* 47(6):994–1001.

Hasler, J. A. (1999). Pharmacogenetics of cytochromes P450. *Mol. Aspects Med.* 20 (1–2):12–24:25–137.

Hattersley, A. T. (1998). Maturity—onset diabetes of the young: clinical heterogeneity explained by genetic heterogeneity. *Diabet. Med.* 15(1):15–24.

Heim, M., Meyer, U. A. (1990). Genotyping of poor metabolisers of debrisoquine by allele-specific PCR amplification. *Lancet* 336(8714):529–532.

Heuring, R. E., Peroutka, S. J. (1987). Characterization of a novel 3H-5-hydroxytryptamine binding site subtype in bovine brain membranes. *J. Neurosci.* 7(3):894–903.

Hietanen, E., Malaveille, C., Camus, A. M., Bereziat, J. C., Brun, G., Castegnaro, M., Michelon, J., Idle, J. R., Bartsch, H. (1986). Interstrain comparison of hepatic and renal microsomal carcinogen metabolism and liver S9-mediated mutagenicity in DA and Lewis rats phenotyped as poor and extensive metabolizers of debrisoquine. *Drug Metab. Dispos.* 14(1):118–126.

Hiroi, T., Imaoka, S., Funae, Y. (1998). Dopamine formation from tyramine by CYP2D6. *Biochem. Biophys. Res. Commun.* 249(3):838–843.

Horai, Y., Nakano, M., Ishizaki, T., Ishikawa, K., Zhou, H. H., Zhou, B. I., Liao, C. L., Zhang, L. M. (1989). Metoprolol and mephenytoin oxidation polymorphisms in Far Eastern Oriental subjects: Japanese versus mainland Chinese. *Clin. Pharmacol. Ther.* 46(2):198–207.

Hoyer, D., Waeber, C., Pazos, A., Probst, A., Palacios, J. M. (1988). Identification of a 5-HT1 recognition site in human brain membranes different from 5-HT1A, 5-HT1B and 5-HT1C sites. *Neurosci. Lett.* 85(3):357–362.

Huber, J. C., Schneeberger, C., Tempfer, C. B. (2002). Genetic modeling of estrogen metabolism as a risk factor of hormone-dependent disorders. *Maturitas* 41 Suppl 1:S55–S64.

Hutzler, J. M., Walker, G. S., Wienkers, L. C. (2003). Inhibition of cytochrome P450 2D6: structure-activity studies using a series of quinidine and quinine analogues. *Chem. Res. Toxicol.* 16(4):450–459.

Idle, J. R., Mahgoub, A., Angelo, M. M., Dring, L. G., Lancaster, R., Smith, R. L. (1979). The metabolism of [14C]-debrisoquine in man. *Br. J. Clin. Pharmacol.* 7(3):257–266.

Idle, J. R., Smith, R. L. (1995). Pharmacogenetics in the new patterns of healthcare delivery. *Pharmacogenetics* 5(6):347–350.

Ingelman-Sundberg, M. (2001). Genetic susceptibility to adverse effects of drugs and environmental toxicants. The role of the CYP family of enzymes. *Mutat. Res.* 482(1–2):11–19.

Ingelman-Sundberg, M., Oscarson, M., McLellan, R. A. (1999). Polymorphic human cytochrome P450 enzymes: an opportunity for individualized drug treatment. *Trends Pharmacol. Sci.* 20(8):342–349.

Johansson, I., Lundqvist, E., Bertilsson, L., Dahl, M. L., Sjoqvist, F., Ingelman-Sundberg, M. (1993). Inherited amplification of an active gene in the cytochrome P450 CYP2D locus as a cause of ultrarapid metabolism of debrisoquine. *Proc. Natl. Acad. Sci. U. S. A.* 90(24):11825–11829.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

Johansson, I., Oscarson, M., Yue, Q. Y., Bertilsson, L., Sjoqvist, F., Ingelman-Sundberg, M. (1994). Genetic analysis of the Chinese cytochrome P4502D locus: characterization of variant CYP2D6 genes present in subjects with diminished capacity for debrisoquine hydroxylation. *Mol. Pharmacol.* 46(3):452–459.

Jones, R. S. (1982). Tryptamine: a neuromodulator or neurotransmitter in mammalian brain? *Prog. Neurobiol.* 19(1–2):117–139.

Jover, R., Bort, R., Gomez-Lechon, M. J., Castell, J. V. (2001). Cytochrome P450 regulation by hepatocyte nuclear factor 4 in human hepatocytes: a study using adenovirus-mediated antisense targeting. *Hepatology* 33(3):668–675.

Kagimoto, M., Heim, M., Kagimoto, K., Zeugin, T., Meyer, U. A. (1990). Multiple mutations of the human cytochrome P450IID6 gene (CYP2D6) in poor metabolizers of debrisoquine. Study of the functional significance of individual mutations by expression of chimeric genes. *J. Biol. Chem.* 265(28):17209–17214.

Kimura, S., Umeno, M., Skoda, R. C., Meyer, U. A., Gonzalez, F. J. (1989). The human debrisoquine 4-hydroxylase (CYP2D) locus: sequence and identification of the polymorphic CYP2D6 gene, a related gene, and a pseudogene. *Am. J. Hum. Genet.* 45(6):889–904.

Kincaid, R. L., McMullin, M. M., Crookham, S. B., Rieders, F. (1990). Report of a fluoxetine fatality. *J. Anal. Toxicol.* 14(5):327–329.

Koyama, E., Chiba, K., Tani, M., Ishizaki, T. (1996). Identification of human cytochrome P450 isoforms involved in the stereoselective metabolism of mianserin enantiomers. *J. Pharmacol. Exp. Ther.* 278(1):21–30.

Koymans, L., Vermeulen, N. P., van Acker, S. A., te Koppele, J. M., Heykants, J. J., Lavrijsen, K., Meuldermans, W., Donne-Op den Kelder, G. M. (1992). A predictive model for substrates of cytochrome P450-debrisoquine (2D6). *Chem. Res. Toxicol.* 5(2):211–219.

Krausz, K. W., Yang, T. J., Gonzalez, F. J., Shou, M., Gelboin, H. V. (1997). Inhibitory monoclonal antibodies to human cytochrome P450 2D6. *Biochem. Pharmacol.* 54(1):15–17.

Kroemer, H. K., Eichelbaum, M. (1995). ''It's the genes, stupid.'' Molecular bases and clinical consequences of genetic cytochrome P450 2D6 polymorphism. *Life Sci.* 56(26):2285–2298.

Kroemer, H. K., Fischer, C., Meese, C. O., Eichelbaum, M. (1991). Enantiomer/enantiomer interaction of (S)- and (R)-propafenone for cytochrome P450IID6-catalyzed 5-hydroxylation: in vitro evaluation of the mechanism. *Mol. Pharmacol.* 40(1):135–142.

Kruger, R., Vieira-Saecker, A. M., Kuhn, W., Berg, D., Muller, T., Kuhnl, N., Fuchs, G. A., Storch, A., Hungs, M., Woitalla, D., Przuntek, H., Epplen, J. T., Schols, L., Riess, O. (1999). Increased susceptibility to sporadic Parkinson's disease by a certain combined alpha-synuclein/apolipoprotein E genotype. *Ann. Neurol.* 45(5):611–617.

Kupfer, A., Schmid, B., Preisig, R., Pfaff, G. (1984). Dextromethorphan as a safe probe for debrisoquine hydroxylation polymorphism. *Lancet* 2(8401):517–518.

Lang, U., Aubert, M. L., Rivest, R. W., Vinas-Bradtke, J. C., Sizonenko, P. C. (1985). Inhibitory action of exogenous melatonin, 5-methoxytryptamine, and 6-hydroxymelatonin on sexual maturation of male rats: activity of 5-methoxytryptamine might be due to its conversion to melatonin. *Biol. Reprod.* 33(3):618–628.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

Leathart, J. B., London, S. J., Steward, A., Adams, J. D., Idle, J. R., Daly, A. K. (1998). CYP2D6 phenotype-genotype relationships in African–Americans and caucasians in Los Angeles. *Pharmacogenetics* 8(6):529–541.

Leino, M., Airaksinen, M. M. (1985). Methoxyindoles of the retina. *Med. Biol.* 63(4):160–169.

Lennard, M. S., Silas, J. H., Freestone, S., Ramsay, L. E., Tucker, G. T., Woods, H. F. (1982a). Oxidation phenotype—a major determinant of metoprolol metabolism and response. *N. Engl. J. Med.* 307(25):1558–1560.

Lennard, M. S., Silas, J. H., Freestone, S., Trevethick, J. (1982b). Defective metabolism of metoprolol in poor hydroxylators of debrisoquine. *Br. J. Clin. Pharmacol.* 14(2):301–303.

Lewis, R. V., Lennard, M. S., Jackson, P. R., Tucker, G. T., Ramsay, L. E., Woods, H. F. (1985). Timolol and atenolol: relationships between oxidation phenotype, pharmacokinetics and pharmacodynamics. *Br. J. Clin. Pharmacol.* 19(3):329–333.

Libby, R. T., Smith, R. S., Savinova, O. V., Zabaleta, A., Martin, J. E., Gonzalez, F. J., John, S. W. (2003). Modification of ocular defects in mouse developmental glaucoma models by tyrosinase. *Science* 299(5612):1578–1581.

Lightfoot, T., Ellis, S. W., Mahling, J., Ackland, M. J., Blaney, F. E., Bijloo, G. J., De Groot, M. J., Vermeulen, N. P., Blackburn, G. M., Lennard, M. S., Tucker, G. T. (2000). Regioselective hydroxylation of debrisoquine by cytochrome P4502D6: implications for active site modelling. *Xenobiotica* 30(3):219–233.

Linnet, K., Olesen, O. V. (1997). Metabolism of clozapine by cDNA-expressed human cytochrome P450 enzymes. *Drug Metab. Dispos.* 25(12):1379–1382.

Llerena, A., Cobaleda, J., Benitez, J. (1989). Debrisoquine hydroxylation phenotypes in healthy volunteers. *Lancet* 1(8651):1398.

Llerena, A., Edman, G., Cobaleda, J., Benitez, J., Schalling, D., Bertilsson, L. (1993). Relationship between personality and debrisoquine hydroxylation capacity. Suggestion of an endogenous neuroactive substrate or product of the cytochrome P4502D6. *Acta Psychiatr. Scand.* 87(1):23–28.

Lovlie, R., Daly, A. K., Matre, G. E., Molven, A., Steen, V. M. (2001). Polymorphisms in CYP2D6 duplication-negative individuals with the ultrarapid metabolizer phenotype: a role for the CYP2D6*35 allele in ultrarapid metabolism? *Pharmacogenetics* 11(1):45–55.

Mahgoub, A., Idle, J. R., Dring, L. G., Lancaster, R., Smith, R. L. (1977). Polymorphic hydroxylation of Debrisoquine in man. *Lancet* 2(8038):584–586.

Maraganore, D. M., Farrer, M. J., Hardy, J. A., McDonnell, S. K., Schaid, D. J., Rocca, W. A. (2000). Case-control study of debrisoquine 4-hydroxylase, *N*-acetyltransferase 2, and apolipoprotein E gene polymorphisms in Parkinson's disease. *Mov. Disord.* 15(4):714–719.

Marez, D., Legrand, M., Sabbagh, N., Guidice, J. M., Spire, C., Lafitte, J. J., Meyer, U. A., Broly, F. (1997). Polymorphism of the cytochrome P450 CYP2D6 gene in a European population: characterization of 48 mutations and 53 alleles, their frequencies and evolution. *Pharmacogenetics* 7(3):193–202.

Martinez, C., Agundez, J. A., Gervasini, G., Martin, R., Benitez, J. (1997). Tryptamine: a possible endogenous substrate for CYP2D6. *Pharmacogenetics* 7(2):85–93.

Masimirembwa, C., Persson, I., Bertilsson, L., Hasler, J., Ingelman-Sundberg, M. (1996).

A novel mutant variant of the CYP2D6 gene (CYP2D6*17) common in a black African population: association with diminished debrisoquine hydroxylase activity. *Br. J. Clin. Pharmacol.* 42(6):713–719.

Masubuchi, Y., Iwasa, T., Hosokawa, S., Suzuki, T., Horie, T., Imaoka, S., Funae, Y., Narimatsu, S. (1997). Selective deficiency of debrisoquine 4-hydroxylase activity in mouse liver microsomes. *J. Pharmacol. Exp. Ther.* 282(3):1435–1441.

Mautz, D. S., Nelson, W. L., Shen, D. D. (1995). Regioselective and stereoselective oxidation of metoprolol and bufuralol catalyzed by microsomes containing cDNA-expressed human P4502D6. *Drug Metab. Dispos.* 23(4):513–517.

McBride, M. C. (2000). Bufotenine: toward an understanding of possible psychoactive mechanisms. *J. Psychoact. Drugs* 32(3):321–331.

McCann, S. J., Pond, S. M., James, K. M., Le Couteur, D. G. (1997). The association between polymorphisms in the cytochrome P-450 2D6 gene and Parkinson's disease: a case-control study and meta-analysis. *J. Neurol. Sci.* 153(1):50–53.

McFayden, M. C., Melvin, W. T., Murray, G. I. (1998). Regional distribution of individual forms of cytochrome P450 mRNA in normal adult human brain. *Biochem. Pharmacol.* 55(6):825–830.

McLellan, R. A., Oscarson, M., Seidegard, J., Evans, D. A., Ingelman-Sundberg, M. (1997). Frequent occurrence of CYP2D6 gene duplication in Saudi Arabians. *Pharmacogenetics* 7(3):187–191.

Mellstrom, B., Bertilsson, L., Lou, Y. C., Sawe, J., Sjoqvist, F. (1983). Amitriptyline metabolism: relationship to polymorphic debrisoquine hydroxylation. *Clin. Pharmacol. Ther.* 34(4):516–520.

Meyer, U. A., Gut, J., Kronbach, T., Skoda, C., Meier, U. T., Catin, T., Dayer, P. (1986). The molecular mechanisms of two common polymorphisms of drug oxidation—evidence for functional changes in cytochrome P-450 isozymes catalysing bufuralol and mephenytoin oxidation. *Xenobiotica* 16(5):449–464.

Miksys, S., Rao, Y., Hoffmann, E., Mash, D. C., Tyndale, R. F. (2002). Regional and cellular expression of CYP2D6 in human brain: higher levels in alcoholics. *J. Neurochem.* 82(6):1376–1387.

Miller, G. P., Hanna, I. H., Nishimura, Y., Guengerich, F. P. (2001). Oxidation of phenethylamine derivatives by cytochrome P450 2D6: the issue of substrate protonation in binding and catalysis. *Biochemistry* 40(47):14215–14223.

Nadir, H. H., Al-Dabbagh, S. G., Idle, J. R. (1982). Elevated serum cholesterol in drug-oxidation-deficient rats. *Biochem. Pharmacol.* 31(8):1665–1668.

Nakamura, K., Goto, F., Ray, W. A., McAllister, C. B., Jacqz, E., Wilkinson, G. R., Branch, R. A. (1985). Interethnic differences in genetic polymorphism of debrisoquin and mephenytoin hydroxylation between Japanese and Caucasian populations. *Clin. Pharmacol. Ther.* 38(4):402–408.

Narasimhachari, N., Heller, B., Spaide, J., Haskovec, L., Fujimori, M., Tabushi, K., Himwich, H. E. (1971). Urinary studies of schizophrenics and controls. *Biol. Psychiatry* 3(1):9–20.

Nebert, D. W. (1997). Polymorphisms in drug-metabolizing enzymes: what is their clinical relevance and why do they exist? *Am. J. Hum. Genet.* 60(2):265–271.

Nebert, D. W., Russell, D. W. (2002). Clinical importance of the cytochromes P450. *Lancet* 360(9340):1155–1162.

Nelson, D. R., Koymans, L., Kamataki, T., Stegeman, J. J., Feyereisen, R., Waxman, D.

J., Waterman, M. R., Gotoh, O., Coon, M. J., Estabrook, R. W., Gunsalus, I. C., Nebert, D. W. (1996). P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics* 6(1):1–42.

Nordin, C., Siwers, B., Benitez, J., Bertilsson, L. (1985). Plasma concentrations of nortriptyline and its 10-hydroxy metabolite in depressed patients—relationship to the debrisoquine hydroxylation metabolic ratio. *Br. J. Clin. Pharmacol.* 19(6):832–835.

Oates, N. S., Shah, R. R., Idle, J. R., Smith, R. L. (1982). Genetic polymorphism of phenformin 4-hydroxylation. *Clin. Pharmacol. Ther.* 32(1):81–89.

Oesch, F., Zimmer, A., Glatt, H. R. (1983). Microsomal epoxide hydrolase in different rat strains. *Biochem. Pharmacol.* 32(11):1783–1788.

Olesen, O. V., Linnet, K. (1997a). Hydroxylation and demethylation of the tricyclic antidepressant nortriptyline by cDNA-expressed human cytochrome P-450 isozymes. *Drug Metab. Dispos.* 25(6):740–744.

Olesen, O. V., Linnet, K. (1997b). Metabolism of the tricyclic antidepressant amitriptyline by cDNA-expressed human cytochrome P450 enzymes. *Pharmacology* 55(5):235–243.

Oscarson, M., Hidestrand, M., Johansson, I., Ingelman-Sundberg, M. (1997). A combination of mutations in the CYP2D6*17 (CYP2D6Z) allele causes alterations in enzyme function. *Mol. Pharmacol.* 52(6):1034–1040.

Ott, J. (2001). Pharmanopo-psychonautics: human intranasal, sublingual, intrarectal, pulmonary and oral pharmacology of bufotenine. *J. Psychoact. Drugs* 33(3):273–281.

Otton, S. V., Crewe, H. K., Lennard, M. S., Tucker, G. T., Woods, H. F. (1988). Use of quinidine inhibition to define the role of the sparteine/debrisoquine cytochrome P450 in metoprolol oxidation by human liver microsomes. *J. Pharmacol. Exp. Ther.* 247(1):242–247.

Paine, M. J., McLaughlin, L. A., Flanagan, J. U., Kemp, C. A., Sutcliffe, M. J., Roberts, G. C., Wolf, C. R. (2003). Residues glutamate 216 and aspartate 301 are key determinants of substrate specificity and product regioselectivity in cytochrome P450 2D6. *J. Biol. Chem.* 278(6):4021–4027.

Payami, H., Lee, N., Zareparsi, S., Gonzales McNeal, M., Camicioli, R., Bird, T. D., Sexton, G., Gancher, S., Kaye, J., Calhoun, D., Swanson, P. D., Nutt, J. (2001). Parkinson's disease, CYP2D6 polymorphism, and age. *Neurology* 56(10):1363–1370.

Peroutka, S. J. (1986). Pharmacological differentiation and characterization of 5-HT1A, 5-HT1B, and 5-HT1C binding sites in rat frontal cortex. *J. Neurochem.* 47(2):529–540.

Raghuram, T. C., Koshakji, R. P., Wilkinson, G. R., Wood, A. J. (1984). Polymorphic ability to metabolize propranolol alters 4-hydroxypropranolol levels but not beta blockade. *Clin. Pharmacol. Ther.* 36(1):51–56.

Raimundo, S., Fischer, J., Eichelbaum, M., Griese, E. U., Schwab, M., Zanger, U. M. (2000). Elucidation of the genetic basis of the common 'intermediate metabolizer' phenotype for drug oxidation by CYP2D6. *Pharmacogenetics* 10(7):577–581.

Ramamoorthy, Y., Yu, A. M., Suh, N., Haining, R. L., Tyndale, R. F., Sellers, E. M. (2002). Reduced (±)-3,4-methylenedioxymethamphetamine (''Ecstasy'') metabolism with cytochrome P450 2D6 inhibitors and pharmacogenetic variants in vitro. *Biochem. Pharmacol.* 63(12):2111–2119.

Raynaud, F., Pevet, P. (1991a). Determination of 5-methoxyindoles in pineal gland and plasma samples by high-performance liquid chromatography with electrochemical detection. *J. Chromatogr.* 564(1):103–113.

Raynaud, F., Pevet, P. (1991b). Effect of different photoperiods on the diurnal rhythm of 5-methoxytryptamine in the pineal gland of golden hamsters (*Mesocricetus auratus*). *J. Neural Transm. Gen. Sect.* 83(3):235–242.

Ritchie, J. C., Idle, J. R. (1982). Population studies of polymorphism in drug oxidation and its relevance to carcinogenesis. In: Armstrong, B., Bartsch, H., eds. *Host Factors in Human Carcinogenesis.* IARC Sci. Publ., pp. 381–394.

Rochat, B., Amey, M., Gillet, M., Meyer, U. A., Baumann, P. (1997). Identification of three cytochrome P450 isozymes involved in *N*-demethylation of citalopram enantiomers in human liver microsomes. *Pharmacogenetics* 7(1):1–10.

Rogawski, M. A., Roth, R. H., Aghajanian, G. K. (1979). Melatonin: deacetylation to 5-methoxytryptamine by liver but not brain aryl acylamidase. *J. Neurochem.* 32(4):1219–1226.

Rowland, K., Ellis, S. W., Lennard, M. S., Tucker, G. T. (1996). Variable contribution of CYP2D6 to the N-dealkylation of S-(-)-propranolol by human liver microsomes. *Br. J. Clin. Pharmacol.* 42(3):390–393.

Ryffel, G. U. (2001). Mutations in the human genes encoding the transcription factors of the hepatocyte nuclear factor (HNF)1 and HNF4 families: functional and pathological consequences. *J. Mol. Endocrinol.* 27(1):11–29.

Sachse, C., Brockmoller, J., Bauer, S., Roots, I. (1997). Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. *Am. J. Hum. Genet.* 60(2):284–295.

Sallee, F. R., DeVane, C. L., Ferrell, R. E. (2000). Fluoxetine-related death in a child with cytochrome P-450 2D6 genetic deficiency. *J. Child Adolesc. Psychopharmacol.* 10(1):27–34.

Saxena, R., Shaw, G. L., Relling, M. V., Frame, J. N., Moir, D. T., Evans, W. E., Caporaso, N., Weiffenbach, B. (1994). Identification of a new variant CYP2D6 allele with a single base deletion in exon 3 and its association with the poor metabolizer phenotype. *Hum. Mol. Genet.* 3(6):923–926.

Schmid, B., Bircher, J., Preisig, R., Kupfer, A. (1985). Polymorphic dextromethorphan metabolism: co-segregation of oxidative *O*-demethylation with debrisoquin hydroxylation. *Clin. Pharmacol. Ther.* 38(6):618–624.

Schoeffter, P., Waeber, C., Palacios, J. M., Hoyer, D. (1988). The 5-hydroxytryptamine 5-HT1D receptor subtype is negatively coupled to adenylate cyclase in calf substantia nigra. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 337(6):602–608.

Sellers, E. M., Tyndale, R. F. (2000). Mimicking gene defects to treat drug dependence. *Ann. N.Y. Acad. Sci.* 909:233–246.

Sellers, E. M., Otton, S. V., Tyndale, R. F. (1997). The potential role of the cytochrome P-450 2D6 pharmacogenetic polymorphism in drug abuse. *NIDA Res. Monogr.* 173:6–26.

Shimada, T., Tsumura, F., Yamazaki, H., Guengerich, F. P., Inoue, K. (2001). Characterization of (±)-bufuralol hydroxylation activities in liver microsomes of Japanese and Caucasian subjects genotyped for CYP2D6. *Pharmacogenetics* 11(2):143–156.

Siddoway, L. A., Thompson, K. A., McAllister, C. B., Wang, T., Wilkinson, G. R.,

Roden, D. M., Woosley, R. L. (1987). Polymorphism of propafenone metabolism and disposition in man: clinical and pharmacokinetic consequences. *Circulation* 75(4):785–791.

Siegle, I., Fritz, P., Eckhardt, K., Zanger, U. M., Eichelbaum, M. (2001). Cellular localization and regional distribution of CYP2D6 mRNA and protein expression in human brain. *Pharmacogenetics* 11(3):237–245.

Sindrup, S. H., Brosen, K., Gram, L. F., Hallas, J., Skjelbo, E., Allen, A., Allen, G. D., Cooper, S. M., Mellows, G., Tasker, T. C., Zussman, B. D. (1992). The relationship between paroxetine and the sparteine oxidation polymorphism. *Clin. Pharmacol. Ther.* 51(3):278–287.

Sohn, D. R., Shin, S. G., Park, C. W., Kusaka, M., Chiba, K., Ishizaki, T. (1991). Metoprolol oxidation polymorphism in a Korean population: comparison with native Japanese and Chinese populations. *Br. J. Clin. Pharmacol.* 32(4):504–507.

Steen, V. M., Molven, A., Aarskog, N. K., Gulbrandsen, A. K. (1995). Homologous unequal cross-over involving a 2.8 kb direct repeat as a mechanism for the generation of allelic variants of human cytochrome P450 CYP2D6 gene. *Hum. Mol. Genet.* 4(12):2251–2257.

Strobl, G. R., von Kruedener, S., Stockigt, J., Guengerich, F. P., Wolff, T. (1993). Development of a pharmacophore for inhibition of human liver cytochrome P-450 2D6: molecular modeling and inhibition studies. *J. Med. Chem.* 36(9):1136–1145.

Su, P., Coutts, R. T., Baker, G. B., Daneshtalab, M. (1993). Analysis of imipramine and three metabolites produced by isozyme CYP2D6 expressed in a human cell line. *Xenobiotica* 23(11):1289–1298.

Sugden, D., Pickering, H., Teh, M. T., Garratt, P. J. (1997). Melatonin receptor pharmacology: toward subtype specificity. *Biol. Cell* 89(8):531–537.

Sullivan, J. P., McDonnell, L., Hardiman, O. M., Farrell, M. A., Phillips, J. P., Tipton, K. F. (1986). The oxidation of tryptamine by the two forms of monoamine oxidase in human tissues. *Biochem. Pharmacol.* 35(19):3255–3260.

Szklarz, G. D., Halpert, J. R. (1998). Molecular basis of P450 inhibition and activation: implications for drug development and drug therapy. *Drug Metab. Dispos.* 26(12):1179–1184.

Tang, W., Stearns, R. A. (2001). Heterotropic cooperativity of cytochrome P450 3A4 and potential drug–drug interactions. *Curr. Drug Metab.* 2(2):185–198.

Tateishi, T., Chida, M., Ariyoshi, N., Mizorogi, Y., Kamataki, T., Kobayashi, S. (1999). Analysis of the CYP2D6 gene in relation to dextromethorphan *O*-demethylation capacity in a Japanese population. *Clin. Pharmacol. Ther.* 65(5):570–575.

Teh, L. K., Ismail, R., Yusoff, R., Hussein, A., Isa, M. N., Rahman, A. R. (2001). Heterogeneity of the CYP2D6 gene among Malays in Malaysia. *J. Clin. Pharm. Ther.* 26(3):205–211.

Tsou, A. P., Kosaka, A., Bach, C., Zuppan, P., Yee, C., Tom, L., Alvarez, R., Ramsey, S., Bonhaus, D. W., Stefanich, E., Jakeman, L., Eglen, R. M., Chan, H. W. (1994). Cloning and expression of a 5-hydroxytryptamine7 receptor positively coupled to adenylyl cyclase. *J. Neurochem.* 63(2):456–464.

Tyndale, R., Aoyama, T., Broly, F., Matsunaga, T., Inaba, T., Kalow, W., Gelboin, H. V., Meyer, U. A., Gonzalez, F. J. (1991). Identification of a new variant CYP2D6 allele lacking the codon encoding Lys-281: possible association with the poor metabolizer phenotype. *Pharmacogenetics* 1(1):26–32.

van Benthem, J., Mans, D. R., Ebels, I., Balemans, M. G. (1985). Rhythmic synthesis of various 5-methoxyindoles in the pineal gland of male adult golden hamsters, kept under the same artificial conditions throughout the year. *J. Neural Transm.* 61(3–4):219–237.

van der Horst, C. J., Ebels, I. (1980). Extraction of pineal and uterine tissue at different pH values: a preliminary report on the occurrence of a few groups of compounds in both tissues. *Cytobios* 29(115–116):191–203.

Voirol, P., Jonzier-Perey, M., Porchet, F., Reymond, M. J., Janzer, R. C., Bouras, C., Strobel, H. W., Kosel, M., Eap, C. B., Baumann, P. (2000). Cytochrome P-450 activities in human and rat brain microsomes. *Brain Res.* 855(2):235–243.

Wainscott, D. B., Cohen, M. L., Schenck, K. W., Audia, J. E., Nissen, J. S., Baez, M., Kursar, J. D., Lucaites, V. L., Nelson, D. L. (1993). Pharmacological characteristics of the newly cloned rat 5-hydroxytryptamine2F receptor. *Mol. Pharmacol.* 43(3):419–426.

Wan, Y. J., Poland, R. E., Han, G., Konishi, T., Zheng, Y. P., Berman, N., Lin, K. M. (2001). Analysis of the CYP2D6 gene polymorphism and enzyme activity in African–Americans in southern California. *Pharmacogenetics* 11(6):489–499.

Wang, T., Roden, D. M., Wolfenden, H. T., Woosley, R. L., Wood, A. J., Wilkinson, G. R. (1984). Influence of genetic polymorphism on the metabolism and disposition of encainide in man. *J. Pharmacol. Exp. Ther.* 228(3):605–611.

Wennerholm, A., Johansson, I., Massele, A. Y., Lande, M., Alm, C., Aden-Abdi, Y., Dahl, M. L., Ingelman-Sundberg, M., Bertilsson, L., Gustafsson, L. L. (1999). Decreased capacity for debrisoquine metabolism among black Tanzanians: analyses of the CYP2D6 genotype and phenotype. *Pharmacogenetics* 9(6):707–714.

Wienkers, L. C. (2001). Problems associated with in vitro assessment of drug inhibition of CYP3A4 and other P-450 enzymes and its impact on drug discovery. *J. Pharmacol. Toxicol. Methods* 45(1):79–84.

Wolf, C. R., Smith, G. (1999). Pharmacogenetics. *Br. Med. Bull.* 55(2):366–386.

Wolf, C. R., Smith, G., Smith, R. L. (2000). Science, medicine, and the future: pharmacogenetics. *BMJ* 320(7240):987–990.

Wolff, T., Distlerath, L. M., Worthington, M. T., Guengerich, F. P. (1987). Human liver debrisoquine 4-hydroxylase: test for specificity toward various monooxygenase substrates and model of the active site. *Arch. Toxicol.* 60(1–3):89–90.

Wrighton, S. A., Schuetz, E. G., Thummel, K. E., Shen, D. D., Korzekwa, K. R., Watkins, P. B. (2000). The human CYP3A subfamily: practical considerations. *Drug Metab. Rev.* 32(3–4):339–361.

Wrighton, S. A., VandenBranden, M., Ring, B. J. (1996). The human drug metabolizing cytochromes P450. *J. Pharmacokinet. Biopharm.* 24(5):461–473.

Yokota, H., Tamura, S., Furuya, H., Kimura, S., Watanabe, M., Kanazawa, I., Kondo, I., Gonzalez, F. J. (1993). Evidence for a new variant CYP2D6 allele CYP2D6J in a Japanese population associated with lower in vivo rates of sparteine metabolism. *Pharmacogenetics* 3(5):256–263.

Yoshimoto, K., Echizen, H., Chiba, K., Tani, M., Ishizaki, T. (1995). Identification of human CYP isoforms involved in the metabolism of propranolol enantiomers—*N*-desisopropylation is mediated mainly by CYP1A2. *Br. J. Clin. Pharmacol.* 39(4):421–431.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Yu, A., Haining, R. L. (2001a). Comparative contribution to dextromethorphan metabolism by cytochrome P450 isoforms in vitro: can dextromethorphan be used as a dual probe for both CTP2D6 and CYP3A activities? *Drug Metab. Dispos.* 29(11):1514–1520.

Yu, A., Haining, R. L. (2001b). Purification, biochemical characterization and comparative enzyme kinetics of recombinant human CYP2D6 1 and CYP2D6 2 variants. *Adv. Exp. Med. Biol.* 500:327–330.

Yu, A., Dong, H., Lang, D., Haining, R. L. (2001). Characterization of dextromethorphan *O*- and *N*-demethylation catalyzed by highly purified recombinant human CYP2D6. *Drug Metab. Dispos.* 29(11):1362–1365.

Yu, A., Kneller, B. M., Rettie, A. E., Haining, R. L. (2002). Expression, purification, biochemical characterization, and comparative function of human cytochrome P450 2D6.1, 2D6.2, 2D6.10, and 2D6.17 allelic isoforms. *J. Pharmacol. Exp. Ther.* 303(3):1291–1300.

Yu, A. M., Granvil, C. P., Haining, R. L., Krausz, K. W., Corchero, J., Kupfer, A., Idle, J. R., Gonzalez, F. J. (2003a). The relative contribution of monoamine oxidase and cytochrome p450 isozymes to the metabolic deamination of the trace amine tryptamine. *J. Pharmacol. Exp. Ther.* 304(2):539–546.

Yu, A. M., Idle, J. R., Byrd, L. G., Krausz, K. W., Kupfer, A., Gonzalez, F. J. (2003b). Regeneration of serotonin from 5-methoxytryptamine by polymorphic human CYP2D6. *Pharmacogenetics* 13(3):173–181.

Yu, A. M., Idle, J. R., Herraiz, T., Kupfer, A., Gonzalez, F. J. (2003c). Screening for endogenous substrates reveals that CYP2D6 is a 5-methoxyindolethylamine *O*-demethylase. *Pharmacogenetics* 13(6):307–319.

Yu, A. M., Idle, J. R., Krausz, K. W., Kupfer, A., Gonzalez, F. J. (2003d). Contribution of individual cytochrome P450 isozymes to the *O*-demethylation of the psychotropic beta-carboline alkaloids harmaline and harmine. *J. Pharmacol. Exp. Ther.* 305(1):315–322.

Yue, Q. Y., Svensson, J. O., Alm, C., Sjoqvist, F., Sawe, J. (1989). Codeine *O*-demethylation co-segregates with polymorphic debrisoquine hydroxylation. *Br. J. Clin. Pharmacol.* 28(6):639–645.

Zanger, U. M., Fischer, J., Raimundo, S., Stuven, T., Evert, B. O., Schwab, M., Eichelbaum, M. (2001). Comprehensive analysis of the genetic factors determining expression and function of hepatic CYP2D6. *Pharmacogenetics* 11(7):573–585.

Zareparsi, S., Kaye, J., Camicioli, R., Grimslid, H., Oken, B., Litt, M., Nutt, J., Bird, T., Schellenberg, G., Payami, H. (1997). Modulation of the age at onset of Parkinson's disease by apolipoprotein E genotypes. *Ann. Neurol.* 42(4):655–658.

Zawilska, J. B., Nowak, J. Z. (1996). Characterization of melatonin receptors in the brain of four avian species: duck, goose, pigeon, and turkey. *Gen. Comp. Endocrinol.* 101(2):227–234.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Request Permission or Order Reprints Instantly!