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

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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, exhibit 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-
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 antiarrhythmics, 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
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 (Batham 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.

**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.
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;
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 $CYP2D$ 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 $CYP2D6*2B$ (Akllillu 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% (Akllillu 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 (Batham et al., 1998), is reported to be present at relatively high frequency among Saudi Arabians (20%) (McLellan et al., 1997) and Ethiopians (29%) (Akllillu 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 (Akllillu et al., 1996; Dahl et al., 1995; Johansson et al., 1993). More recently, $CYP2D6*35$ (Lovlie et al., 2001) was identified in Caucasian UM 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.
Table 1. Drug substrates and their metabolic pathways catalyzed by CYP2D6 and selected CYP2D6 inhibitors.

| Psychotropic drugs         | Reaction                                      | Reference                                                                 |
|----------------------------|-----------------------------------------------|---------------------------------------------------------------------------|
| Amitriptyline              | Benzylid 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              | Benzylid 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)       |
| Drug                  | Metabolism                         | References                                      |
|----------------------|------------------------------------|------------------------------------------------|
| Encainide            | O-demethylation                     | (Wang et al., 1984)                             |
| Flecaïnide           | O-dealkylation                      | (Beckmann et al., 1988)                         |
| Metoprolol           | Benzylic hydroxylation and         | (Lennard et al., 1982a,b; Mautz et al., 1995)  |
|                      | O-demethylation                     |                                                |
| 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             | (Lightfoot et al., 2000; Mahgoub et al., 1977;  |
|                      | hydroxylation                       | 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 |                        |                                                 |

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(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-HT₃ 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-methylenedioxyamphetamine (“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
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 (Coulls 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.
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
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 B1 (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 EcoRI (E) and BamHI (B) are depicted. Black boxes represent CYP2D6 exons. The bar represents 1 kb. B, Southern blot genotyping of wild-type and Tg-CYP2D6 mice. Tail DNA (15 μg) was digested with BamHI 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 electrophoresis (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.
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](image-url)  
**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 (HNF4a) (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 HNF4a 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**     |                    |                       |                     |
| \(T_{\text{max}}\) (hr) | 2.5 ± 1.8          | 6.7 ± 0.7             | 4.6 ± 1.8           |
| \(C_{\text{max}}\) (nmol/L) | 2940 ± 795         | 879 ± 128\(^b\)       | 467 ± 61\(^b,c\)    |
| AUC\(_0–24\) (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** |                |                       |                     |
| \(T_{\text{max}}\) (hr) | 1.7 ± 0.3          | 3.3 ± 2.3             | 0.8 ± 1.2           |
| \(C_{\text{max}}\) (nmol/L) | 110 ± 12           | 535 ± 79\(^b\)        | 1080 ± 97\(^b,c\)   |
| AUC\(_0–24\) (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.
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,
### 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 |
| Bufuralol | $1'$-Hydroxylation | $1'$-Hydroxybufuralol | Yeast | 1.3 ± 0.2 | (Bapiro et al., 2002) |
| | | | Insect cells, purified | 1.9 ± 0.3 | (Yu et al., 2001) |
| | | | Yeast | 18.5 ± 7.3 | (Evert et al., 1997) |
| Debrisoquine | $4'$-Hydroxylation | $4'$-Hydroxydebrisoquine | Yeast | 55.2 ± 7.1 | (Bapiro et al., 2002) |
| | | | Insect cells, purified | 12.1 ± 1.3 | (Chiu et al., 2001) |
| p-Tyramine | $3'$-Hydroxylation | Dopamine | Yeast | 190 ± 19.5 | (Hiroi et al., 1998) |
| | | | Insect cells, purified | 152 ± 52.3 | (Haining and Yu, 2003) |
| m-Tyramine | $4'$-Hydroxylation | Dopamine | Yeast | 58.2 ± 13.8 | (Hiroi et al., 1998) |
| | | | Insect cells, purified | 163.0 ± 80 | (Haining and Yu, 2003) |
| 3-Methoxyphenethylamine | $O$-Demethylation | 3-Hydroxyphenethylamine | Yeast | 190 ± 19.5 | (Hiroi et al., 1998) |
| 4-Methoxyphenethylamine | $O$-Demethylation | 4-Hydroxyphenethylamine | Yeast | 152 ± 52.3 | (Haining and Yu, 2003) |
| 5-Methoxytryptamine | $O$-Demethylation | 5-Hydroxytryptamine | Yeast | 17.1 ± 3.96 | (Yu et al., 2003b) |
| 5-MDMT | $O$-Demethylation | Bufotenine | Yeast | 12.4 ± 0.35 | (Yu et al., 2003c) |
| Pinoline | $O$-Demethylation | 6-HO-THBC | Yeast | 75.1 ± 10.1 | (Haring and Yu, 2001) |
| Harmaline | $O$-Demethylation | Harmalen | Yeast | 160 ± 17 | (Miller et al., 2001) |
| Harmine | $O$-Demethylation | Harmol | Yeast | 111 ± 5 | (Miller et al., 2003b) |

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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;
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.
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.
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 \(\mu\)g/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 \(\beta\)-carboline formation, either spontaneously (Pictet–Spengler reaction) or from a \(N^\beta\)-methyltetrahydrofolate-dependent reaction (Reaction F).
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.

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