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What is This?
Pharmacogenetics of the Cytochrome P450 Enzyme System: Review of Current Knowledge and Clinical Significance

Amal Al Omari, MS, and Daryl J. Murry, PharmD

Genetic variation in drug metabolizing enzymes is an important contributor to interindividual variation in drug disposition and response and is associated with significant clinical consequences. Many commonly used drugs are dependent on the cytochrome P450 monooxygenase enzymes (CYP450) for their metabolism and elimination. At present, more than 57 active human CYP450 genes are known, and the majority of these genes are polymorphic. Despite the large number of CYP450 genes, only the CYP1, CYP2, and CYP3 families of enzymes have a major role in drug metabolism. Approximately 10 CYP450s are responsible for the metabolism of a large number of pharmacologic agents in human beings. The polymorphic forms of the CYP450s are responsible for the development of a significant number of adverse drug reactions and may also contribute to drug response. Genetic polymorphisms have now been identified in the genes encoding all the main CYP450s that contribute to drug and other xenobiotic metabolism, and there are marked interethnic differences in the distribution and frequency of variant alleles. A review of the progress in the pharmacogenetics of P450s that are important for drug metabolism is presented with particular emphasis on the clinical relevance of this research.

KEY WORDS: Cytochrome P450, pharmacogenetics, genetic polymorphism, drug-metabolizing enzymes, poor metabolizer.

INTERPATIENT VARIABILITY in response to drug therapy is the rule, not the exception, for almost all medications.1 It is well recognized that individuals may respond quite differently to the same dose or even the same plasma concentration of a drug.2 Potential causes for such variability in drug effects include the pathogenesis and severity of the disease being treated, drug interactions, and the individual’s age, nutritional status, organ function, and concomitant diseases.3 In many cases, however, genetic factors may have an even greater influence on drug efficacy and toxicity.4

The term pharmacogenetics was first coined by Vogel in 1959, and since then it has been used to refer to the effects of genetic differences on a person’s response to drugs.5 Pharmacogenetics can help in optimizing drug efficacy and minimizing adverse drug reactions through identification of those who are likely to respond or not respond to the medication and those who are at increased risk for adverse reactions to drugs based on their genetic makeup.6

Genetic variation in drug-metabolizing enzymes is an important contributor to interindividual differences in drug disposition and is associated with significant clinical consequences.2 Many commonly used drugs are dependent on cytochrome P450 monooxygenase enzymes (CYP450s) for their metabolism and/or elimination.4 More than 80% of all phase I-dependent metabolism of clinically used drugs is carried out by the CYP450s,13 and these...
enzymes also metabolize a large number of chemicals. The polymorphic forms of CYP450s are responsible for the development of a significant number of adverse drug reactions and may also contribute to nonresponsiveness to drug therapy. The purpose of this article is to review progress in the pharmacogenetics of CYP450s that are important for drug metabolism with particular emphasis on the clinical relevance of this research.

CYTOCHROME P450s

The CYP450s are a superfamily of heme-containing enzymes that are responsible for the phase I-dependent metabolism of drugs and other chemicals. The name cytochrome P450 derives from the spectroscopic observation that when a drug is bound to the reduced-heme enzyme (Fe$^{2+}$), carbon monoxide can bind to the complex and absorb light at a characteristic and distinctive 450 nm. There is extensive interindividual variation in human drug metabolism. A number of factors contribute to the variation in CYP450 activity, including: (1) genetic polymorphisms, (2) environmental factors, (3) physiological status, and (4) disease state. This variation in CYP enzyme activity is responsible for the occurrence of adverse effects or lack of therapeutic efficacy in many cases.

To add uniformity to CYP450 classification, CYP proteins are arranged into families and subfamilies on the basis of amino acid sequence homology, according to a standard nomenclature system adapted in 1996. Enzymes that share at least 40% sequence homology are assigned to a family designated by an Arabic numeral, whereas those sharing at least 55% homology makeup a particular subfamily designated by a letter. Single members of a subfamily represent a particular enzyme and are designated by the number following the subfamily description (eg, CYP2D6). For each enzyme, the most common or “wild-type” allele is denoted as *1, and allelic variants are sequentially numbered as they are identified (ie, *2, *3, etc.). The wild-type allele usually denotes normal enzyme activity; however, this is not always the case.

At present, more than 57 active human P450 genes and 58 pseudogenes are known. The majority of genes are polymorphic, and current information on genetic variants can be found at the human CYP allele home page (http://www.imm.ki.se/CYPalleles/). More than 434 different alleles of the genes encoding xenobiotic metabolizing P450 enzymes are presented on this site. However, despite the large number of CYP genes and enzymes, it appears that only the CYP1, CYP2, and CYP3 families of enzymes have a major role in drug metabolism. Other CYP families have essential roles in intermediary metabolism and the metabolism of endogenous substances. The CYP4 family, for example, is involved in the oxidation of fatty acids, arachidonic acid, and eicosanoids.

Overall, approximately 10 cytochrome P450 enzymes are responsible for the metabolism of a large number of pharmacologic agents in human beings (Table 1). CYP450s with known polymorphisms (in particular, CYP2C9, CYP2C19 and CYP2D6) are responsible for approximately 40% of CYP450-mediated drug metabolism, making drug dosing problematic. In general, 4 phenotypes can be identified: poor metabolizers (PMs), who express dysfunctional or inactive enzymes; intermediate metabolizers, who are heterozygous for one deficient allele or carry 2 alleles that cause reduced activity; extensive metabolizers (EMs), who have 2 normal alleles; and ultrarapid metabolizers (UMs), who have larger quantities of expressed enzymes because of gene duplication.

PHARMACOGENETICS OF CYP450

The CYP1 Gene Family

The CYP1 gene family is composed of 3 genes, CYP1A1, CYP1A2, and CYP1B1. Although CYP1A1 and CYP1B1 do not appear to metabolize known drugs, CYP1A2 has a role in the metabolism of several commonly prescribed drugs, including antipsychotics, caffeine, and theophylline.

CYP 1A Subfamily

Cytochrome P450 1A1 is expressed extrahepatically, mainly in lung tissue. More than 10 alleles have been identified for CYP1A1 to date, and several of these alleles give rise to amino acid substitutions. Human CYP1A1 is involved in the activation of major classes of tobacco procarcinogens, including polycyclic aromatic hydrocarbons and aromatic amines. A consistent association between homozygosity for 2 linked polymorphisms (designated as the CYP1A1*2
ability to induce CYP1A.20 However, the functional significance of these polymorphisms is not clear. CYP1A1 is reported to be inducible, and its induction is regulated via the aryl hydrocarbon receptor (AhR), similar to other members of the CYP1 family.11 Approximately 10% of Caucasians have a highly inducible form of the enzyme.16 One study found a significant association between a polymorphism at codon 554 of the AhR and induced CYP1A1 activity in lymphocytes, but no association between activity and variant CYP1A1 alleles was detected. Further studies are needed to fully understand the factors that determine an individual’s ability to induce CYP1A.20

Cytochrome P450 1A2 is expressed mainly in the liver, where it accounts for approximately 13% of total hepatic CYP content.2 In vivo CYP1A2 activity exhibits a significant degree of interindividual variation, which is probably a result of a combination of environmental (induction by tobacco smoke) and genetic (polymorphisms) influences.21 Clearance of many CYP1A2 substrates, including antipsychotics (clozapine and olanzapine), antidepressants (imipramine, fluvoxamine, paroxetine, and sertraline), and theophylline are increased in smokers compared with nonsmokers.22 More than 23 polymorphisms have been reported in the CYP1A2 gene.15 However, polymorphisms in the coding regions are rare, and only 2 genetic variants with important functional effects in CYP1A2 activity, CYP1A2*7 and CYP1A2*11, have been described.21 More common polymorphisms that may be functionally significant have been found in noncoding regions. Smokers carrying variants CYP1A2*1C and CYP1A2*1F have significantly different enzyme activity compared with smokers carrying only the wild-type allele.23

Cytochrome P450 1B1 is predominantly expressed in extrahepatic locations and is frequently overexpressed in solid tumors, which might be useful in designing drugs for chemotherapeutic intervention.12 CYP1B1 has an important role in the metabolism of polyaromatic carcinogens. It also metabolizes steroid hormones,11 especially the 4'-hydroxylation of estradiol. Thus, CYP1B1 gene polymorphisms may play a role in susceptibility to hormone-dependent cancers such as breast, prostate, and endometrial cancers.11,24,25 Rare mutations in this gene have been associated with primary congenital glaucoma, but a number of more common polymorphisms have also been detected.20 Five common nonsynonymous CYP1B1 single nucleotide polymorphisms (SNPs) have been identified, and 7 haplotypes carrying 1 or more of these SNPs have been characterized.11 Two alleles, CYP1B1*6 and CYP1B1*7, appear to be associated with a highly significant decrease in both 2- and 4-hydroxylation of 17 β-estradiol and a decreased capacity to metabolize benzo[α]-pyrene.11,20 Overexpression of CYP1B1 in a Chinese hamster ovary cell line resulted in a significant decrease in sensitivity toward the cytotoxic effects of docetaxel. This effect was reversed by co-incubating the cells with both docetaxel and the CYP1 inhibitor alphaphthoflavone. Thus, overexpression of CYP1B1 has been proposed as a novel mechanism of anticancer drug resistance.20 Overall, CYP1B1 does not play a major role in drug metabolism because of its extrahepatic localization. Nonetheless, CYP1B1 may have a critical role in tissue-specific metabolism of certain drugs and endogenous substances.11

The CYP2 Gene Family

Cytochrome P450 2A6 is expressed mainly in the liver, but it is also expressed in extrahepatic tissue. The CYP2A6 isoenzyme is expressed mainly in the liver, and Chinese populations has been reported.17,18 These polymorphisms give rise to the amino acid substitution Ile462Val, which has also been shown to interact with polymorphism in the myeloperoxidase gene to increase the overall risk of non-small-cell lung cancer.19 However, the functional significance of these polymorphisms is not clear. CYP1A1 is reported to be inducible, and its induction is regulated via the aryl hydrocarbon receptor (AhR), similar to other members of the CYP1 family.11 Approximately 10% of Caucasians have a highly inducible form of the enzyme.16 One study found a significant association between a polymorphism at codon 554 of the AhR and induced CYP1A1 activity in lymphocytes, but no association between activity and variant CYP1A1 alleles was detected. Further studies are needed to fully understand the factors that determine an individual’s ability to induce CYP1A.20

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### Table 2

Cytochrome P450 Common Variant Alleles and Functional Consequences Relevant to Drug Metabolism

<table>
<thead>
<tr>
<th>Enzyme and Allelic Variants</th>
<th>Nucleotide Changes (cDNA or gene)</th>
<th>Effect (eg, amino acid changes, if present)</th>
<th>Enzyme Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP1A2*1C</td>
<td>-3860G&gt;A</td>
<td>None</td>
<td>Decreased inducibility</td>
</tr>
<tr>
<td>CYP1A2*1D</td>
<td>-2467delT</td>
<td>None</td>
<td>Unclear</td>
</tr>
<tr>
<td>CYP1A2*1F</td>
<td>-163C&gt;A</td>
<td>None</td>
<td>Higher inducibility</td>
</tr>
<tr>
<td>CYP1A2*1K</td>
<td>-739T&gt;G; -729C&gt;T; -163C&gt;A</td>
<td>None</td>
<td>Decreased</td>
</tr>
<tr>
<td>CYP1A2*7</td>
<td>3534G&gt;A</td>
<td>Splicing defect</td>
<td>Decreased</td>
</tr>
<tr>
<td>CYP1A2*11</td>
<td>558C&gt;A</td>
<td>Phe186Leu</td>
<td>Decreased</td>
</tr>
<tr>
<td>CYP2A6:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2A6*1B</td>
<td>Gene conversion in the 3' flanking region</td>
<td>Gene conversion</td>
<td>Increased</td>
</tr>
<tr>
<td>CYP2A6*1x2</td>
<td>None</td>
<td>Gene duplication</td>
<td>Increased</td>
</tr>
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<td>Leu160His</td>
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<td>CYP2A6 deleted</td>
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<td>TATA box</td>
<td>Decreased</td>
</tr>
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<td>CYP2A6*11</td>
<td>3391T&gt;C</td>
<td>Ser224Pro</td>
<td>Decreased</td>
</tr>
<tr>
<td>CYP2B6:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2B6*4</td>
<td>18053A&gt;G</td>
<td>Lys262Arg</td>
<td>Increased</td>
</tr>
<tr>
<td>CYP2B6*5</td>
<td>25505C&gt;T</td>
<td>Arg487Cys</td>
<td>Decreased?</td>
</tr>
<tr>
<td>CYP2B6*6</td>
<td>15631G&gt;T;18053A&gt;G</td>
<td>Gln172His; Lys262Arg</td>
<td>Increased</td>
</tr>
<tr>
<td>CYP2B6*7</td>
<td>15631G&gt;T;18053A&gt;G; 25505C&gt;T</td>
<td>Lys262Arg;Arg487Cys</td>
<td>Decreased?</td>
</tr>
<tr>
<td>CYP2B6*16</td>
<td>18053A&gt;G;21011T&gt;C</td>
<td>Ile328Thr;Lys262Arg</td>
<td>Decreased</td>
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<tr>
<td>CYP2C8:</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C8*2</td>
<td>805A&gt;T</td>
<td>Ile269Phe</td>
<td>Decreased</td>
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<tr>
<td>CYP2C8*3</td>
<td>416G&gt;A; 1196A&gt;G</td>
<td>Arg139Lys;Lys399Arg</td>
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<td>CYP2C8*4</td>
<td>792C&gt;G</td>
<td>Ile264Met</td>
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<td>CYP2C8*5</td>
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<tr>
<td>CYP2C9:</td>
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<td></td>
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<tr>
<td>CYP2C9*2</td>
<td>430C&gt;T</td>
<td>Arg144Cys</td>
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</tr>
<tr>
<td>CYP2C9*3</td>
<td>1075A&gt;C</td>
<td>Ile359Leu</td>
<td>Decreased</td>
</tr>
<tr>
<td>CYP2C9*5</td>
<td>1080C&gt;G</td>
<td>Asp360Glu</td>
<td>Decreased</td>
</tr>
<tr>
<td>CYP2C9*6</td>
<td>818delA</td>
<td>Frameshift</td>
<td>None</td>
</tr>
<tr>
<td>CYP2C19:</td>
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<td></td>
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<tr>
<td>CYP2C19*2</td>
<td>681G&gt;A</td>
<td>Splicing defect</td>
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<tr>
<td>CYP2C19*3</td>
<td>636C&gt;A</td>
<td>Premature stop codon</td>
<td>None</td>
</tr>
<tr>
<td>CYP2C19*17</td>
<td>99C&gt;T; 991A&gt;G</td>
<td>Ile331Val</td>
<td>Increased transcriptions</td>
</tr>
<tr>
<td>CYP2D6:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6*2xN</td>
<td>1661G&gt;C; 2850C&gt;T; 4180G&gt;C</td>
<td>Arg296Cys; Ser486Thr</td>
<td>Increased</td>
</tr>
<tr>
<td>(N=2,3,4,5 or13)</td>
<td></td>
<td>(N active genes)</td>
<td></td>
</tr>
<tr>
<td>CYP2D6*3</td>
<td>2549delA</td>
<td>Frameshift</td>
<td>None</td>
</tr>
<tr>
<td>CYP2D6*4</td>
<td>1846C&gt;A</td>
<td>Splicing defect</td>
<td>None</td>
</tr>
<tr>
<td>CYP2D6*5</td>
<td>CYP2D6 deleted</td>
<td>CYP2D6 deleted</td>
<td>None</td>
</tr>
<tr>
<td>CYP2D6*6</td>
<td>1707delT</td>
<td>Frameshift</td>
<td>None</td>
</tr>
<tr>
<td>CYP2D6*10</td>
<td>100C&gt;T; 1661G&gt;C; 4180G&gt;C</td>
<td>Pro34Ser; Ser486Thr</td>
<td>Decreased</td>
</tr>
<tr>
<td>CYP2D6*17</td>
<td>1023C&gt;T; 1661G&gt;C; 2850C&gt;T; 4180G&gt;C</td>
<td>Thr107Ile; Arg296Cys; Ser486Thr</td>
<td>Decreased</td>
</tr>
<tr>
<td>CYP2E1:</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CYP2E1*1D</td>
<td>8 repeats in the 5'-flanking region</td>
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<td>Increased inducibility</td>
</tr>
<tr>
<td>CYP2E1*2</td>
<td>1132G&gt;A</td>
<td>Arg76His</td>
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<tr>
<td>CYP2E1*5</td>
<td>-1293G&gt;C; -1053C&gt;T; 7632T&gt;A</td>
<td>None</td>
<td>Increased</td>
</tr>
</tbody>
</table>

(continued)
CYP2A6 is known to metabolize only a few drugs, including nicotine. However, CYP2A6 is involved in the activation of many toxic and/or tobacco-related procarcinogens. The wide variation in CYP2A6 levels and activity can be attributed largely to polymorphisms in the CYP2A6 gene. To date, more than 20 polymorphisms and 22 allelic variants have been identified. The most common variant allele in Asians is CYP2A6*4, which results in gene deletion. This allele is present at a frequency of 15% to 20% in Asians, whereas it is rare in Europeans, with an allele frequency of approximately 3%. In Caucasians, the most common variant allele is CYP2A6*2, which yields an inactive enzyme. This allele occurs at an allele frequency of approximately 3% in Caucasians, but it has not been detected in Asians. Some individuals also have 2 copies of the wild-type allele (CYP2A6*1/*1 x 2) and may therefore show unusually fast metabolism. Other variant alleles (Table 2) are found at frequencies of less than 2% in studies reported to date. The low variant allele frequency for CYP2A6 suggests that absence of activity is common only in Asians. Considering the important role of CYP2A6 in nicotine and procarcinogen metabolism, CYP2A6 activity may directly impact smoking behavior, risk of tobacco-related cancers, and treatment of nicotine addiction. Japanese carriers of the defective CYP2A6*4 have been shown to have less risk of tobacco-induced lung cancer. In addition, CYP2B6 activates a number of procarcinogens, including aflatoxin and various nitrosamines. Extensive interindividual variability of CYP2B6 expression of up to 100-fold has been observed. There is broad interindividual variability of pharmacokinetic parameters of several CYP2B6 drug substrates in vivo, suggesting significant differences in the systemic exposure to a variety of drugs that are metabolized by CYP2B6. Enzyme induction is mediated via the constitutive androstane receptor and is expected to be an important factor for interindividual differences in expression. Furthermore, CYP2B6 is highly polymorphic, with more than 48 different alleles described to date, and several of these occur at high frequency among Caucasians. Many variant alleles with amino acid substitutions causing functional alterations have been described. Polymorphic variants resulting in reduced expression levels include CYP2B6*5 (Arg487Cys) and CYP2B6*7 (Gln172His; Lys262Arg; Arg487Cys). In addition, CYP2B6*6 has been associated with reduced enzyme expression but higher activity using cyclophosphamide as a substrate. Other studies, however, reported conflicting results using different substrates. The alleles CYP2B6*4, CYP2B6*6, and CYP2B6*7 (which are all associated with a Lys262Arg substitution) encode proteins with higher intrinsic

<table>
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<tr>
<th>Enzyme and Allelic Variants</th>
<th>Nucleotide Changes (cDNA or gene)</th>
<th>Effect (eg, amino acid changes, if present)</th>
<th>Enzyme Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP3A:</td>
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<td></td>
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<tr>
<td>CYP3A4*1B</td>
<td>-392A&gt;G</td>
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<td>Unclear</td>
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<td>CYP3A5*3</td>
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</tr>
<tr>
<td>CYP3A5*5</td>
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<td>Splicing defect</td>
<td>Severely decreased?</td>
</tr>
<tr>
<td>CYP3A5*6</td>
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<td>Severely decreased</td>
</tr>
<tr>
<td>CYP3A5*7</td>
<td>27131_27132insT</td>
<td>Frame shift</td>
<td>Severely decreased?</td>
</tr>
<tr>
<td>CYP3A7*1C</td>
<td>-291G&gt;T; -284T&gt;A; -282T&gt;G; -281A&gt;T; -270T&gt;G; -262T&gt;A; -232A&gt;C</td>
<td>None</td>
<td>Increased Expression</td>
</tr>
</tbody>
</table>

*This table lists examples of CYP450 allelic variants, which are thought to have functional significance, but it is not meant to be exhaustive. A more comprehensive table is found on the Web page for the CYP allele nomenclature committee, from which this table was adapted.

CYP2B6 was first considered of minor importance, since it was thought to constitute a small fraction of total hepatic CYP content and was not detected in all livers. CYP2B6 is now known to constitute about 3% to 5% of the total microsomal P450 pool and is also detected at lower levels in extrahepatic tissues, including human brain, intestine, kidney, and lung. This enzyme plays an important role in the biotransformation of several clinically important drugs, including cyclophosphamide, diazepam, efavirenz, and S-mephenytoin. In addition, CYP2B6 activates a number of procarcinogens, including aflatoxin and various nitrosamines. Extensive interindividual variability of CYP2B6 expression of up to 100-fold has been observed. There is broad interindividual variability of pharmacokinetic parameters of several CYP2B6 drug substrates in vivo, suggesting significant differences in the systemic exposure to a variety of drugs that are metabolized by CYP2B6. Enzyme induction is mediated via the constitutive androstane receptor and is expected to be an important factor for interindividual differences in expression. Furthermore, CYP2B6 is highly polymorphic, with more than 48 different alleles described to date, and several of these occur at high frequency among Caucasians. Many variant alleles with amino acid substitutions causing functional alterations have been described. Polymorphic variants resulting in reduced expression levels include CYP2B6*5 (Arg487Cys) and CYP2B6*7 (Gln172His; Lys262Arg; Arg487Cys). In addition, CYP2B6*6 has been associated with reduced enzyme expression but higher activity using cyclophosphamide as a substrate. Other studies, however, reported conflicting results using different substrates. The alleles CYP2B6*4, CYP2B6*6, and CYP2B6*7 (which are all associated with a Lys262Arg substitution) encode proteins with higher intrinsic
clearance values for 7-ethoxy-4-trifluoromethylcoumarin.13 CYP2B6*16 with Lys262Arg and Ile328Thr substitutions causes decreased expression of the corresponding enzyme and has been shown to influence the in vivo metabolism of efavirenz.34 In addition, owing to the important role of CYP2B6 for cyclophosphamide activation, polymorphisms of this enzyme may affect cyclophosphamide disposition and treatment outcome, however, further studies are still needed to fully characterize their impact.11

CYP 2C Subfamily

The CYP2C subfamily is composed mainly of CYP2C8, CYP2C9, CYP2C18, and CYP2C19, which collectively represent about 20% of total CYP enzymes expressed in the liver.2 The CYP2C enzymes are involved in the metabolism of about 20% of currently used drugs (Table 1). For example, CYP2C19 catalyzes the metabolism of citalopram, cyclophosphamide, diazepam, and omeprazole. CYP2C9 is responsible for the metabolism of warfarin, phenytoin, and the nonsteroidal anti-inflammatory drugs. CYP2C8 plays an important role in about 5% of drugs, especially antidiabetics, antimalarials, paclitaxel, and retinoic acid.35 CYP2C18 does not appear to play a significant role in drug metabolism and probably represents an inactive gene.11

CYP2C8

Cytochrome P450 2C8 is a major hepatic P450, but extrahepatic CYP2C8 is also present in numerous tissues including the kidney, intestine, brain, and heart.35 Several functionally relevant SNPs have been identified in the CYP2C8 gene, and at least 10 variant alleles have been described thus far.15 The most common variant alleles CYP2C8*2 (Ile269Phe) and CYP2C8*3 (Arg139Lys, Lys399Arg) are both associated with reduced in vitro enzyme activity using paclitaxel as a substrate. However, CYP2C8*2 is more frequent in African Americans, whereas CYP2C8*3 is more commonly expressed in Caucasian subjects.35 These polymorphisms might be expected to have important clinical and pathophysiological consequences and could influence the efficacy of drug treatment (eg, paclitaxel), but definitive studies have yet to determine the relevance to metabolism in vivo. Linkage disequilibrium has been reported to exist between CYP2C8*3 and CYP2C9*2,36 and a strong association between carriers of CYP2C8*3 and CYP2C9*2 or *3 alleles and an increased risk of acute myocardial infarction in male subjects has been reported.37 CYP2C8*4 (Ile264Met) has a frequency of about 8% in white subjects, but its impact on enzyme activity is unclear.38 Other allelic variants are rare; of these variants, CYP2C8*5 is associated with a frameshift mutation, which results in an inactive enzyme.35 This allelic variant was suspected to be associated with drug toxicity in a hypercholesterolemic patient treated with cerivastatin.39 However, other studies detected this allele at a frequency of just 0.25% in Japanese subjects.35

CYP2C9

CYP2C9 is the most important member of the YP2C subfamily, as it is the largest contributor among the 4 isoforms in this subfamily to total human liver CYP450 content, and it is involved in the metabolism of a host of clinically important drugs, including the narrow therapeutic index drugs phenytoin and warfarin. CYP2C9 is subject to significant genetic polymorphism, such that up to 40% of Caucasian populations are carriers of alleles that encode partially defective functional forms of the enzyme.40 More than 100 SNPs have been described in the regulatory and coding regions of the CYP2C9 gene, and over 30 variant alleles have been reported to date.15 CYP2C9*2 (Arg144Cys) and CYP2C9*3 (Ile359Leu) are the 2 most common variant alleles in Caucasian subjects, but they are much less prevalent in African Americans and Asians.41 Some CYP2C9 alleles, such as CYP2C9*5, CYP2C9*6, CYP2C9*8 and CYP2C9*11, were detected at a low frequency in only African-derived populations.42 Another rare allele, CYP2C9*4, has been reported in one Japanese patient who had an adverse reaction to phenytoin.40 These alleles, except for the CYP2C9*6, encode enzymes with single amino acid substitutions and reduced activity. CYP2C9*6 contains an adenine base pair deletion at nucleotide 818, which results in a premature stop codon and a truncated inactive protein.43 Clinical studies have shown that individuals carrying at least one variant allele have a reduced CYP2C9 enzyme activity that may be substrate dependent.2 Since CYP2C9*2 and *3 alleles in human beings were discovered first and are much more prevalent, the clinical relevance of these alleles has been more thoroughly investigated. Several studies have shown a significant reduction, 30% to 60% (CYP2C9*2) and greater than 90% (CYP2C9*3) in S-warfarin 7-hydroxylation, which directly affects the warfarin daily dose required to attain a target international normalized ratio.44 In addition, patients with a variant allele have a greater risk of major bleeding events, especially during the initiation of treatment.44 Thus, CYP2C9 genotyping may help identify high-risk
patients who are candidates for lower warfarin doses, more frequent monitoring, or alternative drug treatments.\textsuperscript{43} CYP2C9 polymorphisms also influence the pharmacokinetics of phenytoin, and several examples of adverse drug reactions have been described in patients with defective CYP2C9 alleles.\textsuperscript{44} A CYP2C9 gene-dose effect also has been observed for phenytoin, with patients expressing at least one variant CYP2C9 allele requiring a 30% lower phenytoin maintenance dose than patients with the *1/*1 genotype.\textsuperscript{45} The clearance of many other drugs is also impacted by the CYP2C9 genotype, but clinically important implications for treatment remain unclear.

CYP2C19

Cytochrome P450 2C19 was one of the first enzymes recognized to exhibit genetic polymorphism, and several allelic variants have been identified with wide interethnic differences in the allele frequencies documented in the literature.\textsuperscript{46} CYP2C19 protein is mainly present in the liver, but significant activity has also been identified in the gut wall.\textsuperscript{47} Several inactive genetic variants exist, although 2 (CYP2C19*2 and CYP2C19*3) account for more than 95% of cases of poor metabolism of substrate drugs. The poor-metabolizer phenotype associated with absence of CYP2C19 activity as a result of the presence of 2 variant alleles is much more common among Asians (10%-25%) than Caucasians (1%-3%) or African-Americans (4%).\textsuperscript{48} These 2 common alleles are associated with production of truncated proteins, although absence of activity can also arise as a result of amino acid substitutions.\textsuperscript{13} Other less-common variant alleles, CYP2C19*4 and CYP2C19*8, result in nonfunctional enzymes that affect either expression of the protein or catalytic activity. These variants, together with the *2 and *3 variants, explain almost all (99.74%) PMs of CYP2C19.\textsuperscript{49} One common novel allele (CYP2C19*17) results in higher expression of the enzyme owing to a mutation in the 5'-upstream region recruiting nuclear factor binding resulting in increased transcription.\textsuperscript{49} Polymorphisms in the CYP2C19 gene affect treatment outcomes with substrate drugs. The proton pump inhibitors omeprazole and lansoprazole are predominantly (more than 80%) metabolized by CYP2C19 and are used as part of dual or triple therapy for eradication of Helicobacter pylori infection.\textsuperscript{48} Several studies indicate that the cure rates of this treatment are higher in PMs (100%) as compared with heterozygous (60%) or homozygous (29%) EMs.\textsuperscript{44,46,48} This finding may be explained by the higher accumulation of plasma omeprazole concentrations in PMs, resulting in a greater degree of gastric acid suppression but no greater incidence of adverse effects because of the wide safety margin of these drugs.\textsuperscript{46} In the case of diazepam, another CYP2C19 substrate, there are clear differences in half-life on the basis of genotype, with PMs displaying an elimination half-life twice that of EMs (88 hours vs. 41 hours).\textsuperscript{40} CYP2C19 also catalyzes the activation of the anticancer drugs cyclophosphamide and ifosfamide, and several studies suggest that the presence of inactive CYP2C19 alleles causes a reduction in the metabolic activation of cyclophosphamide, thereby lowering the risk of toxicity but worsening the therapeutic response.\textsuperscript{41} The rapid CYP2C19*17 allele has an allele frequency of about 18% in Caucasians,\textsuperscript{49} and it can be envisioned that this allele could cause a more efficient treatment with cyclophosphamide. However, it may cause therapeutic failure with, for example, proton pump inhibitors or antidepressants that are metabolized by the CYP2C19 enzyme.

CYP2D6

CYP2D6 is one of the most studied human P450 enzymes. It is involved in the metabolism of a large number of drugs used in clinical practice, including antidepressants, antipsychotics, antiarrhythmics, β-blockers, analgesics, and many others.\textsuperscript{2} CYP2D6 was the first CYP450 for which a genetic polymorphism was identified,\textsuperscript{51} and the CYP2D6 gene is now clearly the most polymorphic of all known human CYP450s, with more than 100 different allelic variants identified and characterized.\textsuperscript{15} Many of the allelic variants described result in inactive enzymes; however, CYP2D6 genotyping assays testing for the 7 most common inactive alleles (*3, *4, *5, *6, *7, *8, and *16) predict the poor metabolizer phenotype in Caucasians, with greater than 99% sensitivity.\textsuperscript{52,53} The CYP2D6*4 allele occurs with a frequency of 22% in Caucasians and accounts for more than 75% of the mutant alleles in this population.\textsuperscript{54} The poor-metabolizer phenotype results from inheriting 2 of any of the inactive CYP2D6 alleles or a combination thereof, which is termed compound heterozygosity.\textsuperscript{45} The incidence of PMs is 5% to 10% in Caucasians, 3.5% to 8% in African Americans, and around 1% in Asian populations.\textsuperscript{55} Despite a lower frequency of PMs, Asian and African American populations tend to have reduced CYP2D6 activity compared with Caucasians.\textsuperscript{56,57} In Asians, this reduced CYP2D6 activity is owing to the high frequency of a mutant CYP2D6*10 allele, which is associated with decreased catalytic activity. The frequency of this allele is about 50% in Asian populations, but the frequency is extremely low among Caucasians.\textsuperscript{54} A similar situation applies to African populations, where the mutated allele CYP2D6*17 encoding an enzyme with decreased
activity was identified with a relatively high frequency.\textsuperscript{57} There are 3 fairly population-specific alleles with CYP2D6\textsuperscript{*4} in Caucasians, *10 in Asians, and *17 in Africans. In addition to EMs, a UM phenotype has also been identified and found to result from gene duplication (up to 13 copies of CYP2D6) and is characterized by a faster than average metabolism.\textsuperscript{13} The frequency of subjects having duplicated/multiduplicated genes is highest in Ethiopia and Saudi Arabia, where up to a third of the population displays this genotype.\textsuperscript{9}

CYP2D6 is a noninducible enzyme; thus its genotype offers a high predictability of CYP2D6-mediated metabolism.\textsuperscript{45} The consequence of genetic variation is that, compared with majority of the population (ie, EMs), PMs will exhibit much higher plasma concentrations of a drug, whereas UMs will exhibit much lower plasma drug concentrations. Thus, PMs are more likely to have adverse effects from drugs that are substrates of the isoenzymes, as well as decreased efficacy from drugs requiring CYP2D6-mediated activation. However, UMs may be at risk for therapeutic failure with drugs inactivated by CYP2D6.

Tricyclic antidepressants are almost entirely metabolized by CYP2D6, and the dosage required corresponds closely with the CYP2D6 phenotype, ranging from 30 mg to 50 mg in PMs to 500 mg in UMs for nortriptyline. The UM phenotype was highly overrepresented in patients that were classified as nonresponders to antidepressant therapy in a psychiatric clinic.\textsuperscript{58} Many antipsychotics are metabolized by CYP2D6, and parkinsonian side effects are seen at higher frequencies in PMs.\textsuperscript{58} Thus, genotyping of psychiatric patients for CYP2D6 prior to drug prescription may help to improve treatment outcomes.

The pain-relieving drugs codeine and tramadol are ineffective in CYP2D6 PMs because of an absence of activation to morphine by CYP2D6. On the other hand, UMs experience adverse reactions of codeine activation to morphine by CYP2D6. \textsuperscript{11}

CYP2E1

CYP2E1 is the only member in the CYP2E subfamily, and although its role in drug metabolism is minor, it has received much attention because of its potentially important role in human susceptibility to toxicity and carcinogenicity of industrial and environmental chemicals. The enzyme is constitutively expressed in the liver but is also expressed and induced in the brain after ethanol treatment or ischemia, and it is also distributed in many other tissues.\textsuperscript{31} CYP2E1 metabolizes mainly low molecular weight compounds and is involved in the bioactivation of various protoxicants and procarcinogenic substrates, including halogenated hydrocarbons and alcohols (eg, ethanol). High CYP2E1 activity has been linked to increased susceptibility to chemical toxicity and cancer. Drugs metabolized by CYP2E1 include acetaminophen, chloroxazone, and trimethadione.\textsuperscript{62} CYP2E1 is also one of several CYP450s demonstrated to convert acetaminophen to toxic reactive quinones (NAPQI), which can cause liver damage.\textsuperscript{63} The human CYP2E1 gene appears to be well conserved, probably because of a critical role of the enzyme in gluconeogenesis during conditions of starvation.\textsuperscript{11} Several genetic polymorphisms have been reported, but the majority of these do not appear to have functional significance. CYP2E1*1D is thought to be associated with greater inducibility and higher CYP2E1 activity.\textsuperscript{64} CYP2E1 was reported to be inducted by alcohol, obesity, and nicotine.\textsuperscript{64} This CYP2E1 polymorphism was found to be sufficiently common (approximately 7\% among Caucasians and 31\% among African Americans) to impact susceptibility to CYP2E1-related diseases.\textsuperscript{64} CYP2E1*2, which is associated with less than 40\% of the catalytic activity of the wild-type enzyme, was detected at a low frequency (2.5\%) in a Chinese population but not in other ethnic groups.\textsuperscript{61} Another variant allele, CYP2E1*5 may have a functional significance.\textsuperscript{65} This allele was found to be rare in Caucasians and African Americans (2\%) but relatively common in Asian populations (27\%).\textsuperscript{66} Although in vitro studies reported a 10-fold increase in transcriptional activity associated with this allelic variant, these findings were not confirmed in vivo.\textsuperscript{67} These variants may be
important contributors to interindividual variability in CYP2E1 activity, but additional studies are still needed to further explain the molecular basis of interindividual variation in CYP2E1 expression and ability to induce this enzyme.

CYP 3A Subfamily

Cytochrome P450 3A enzymes are of major importance since they are the most abundantly expressed human CYP isoforms in the liver and small intestine. These enzymes exhibit extremely broad substrate specificity; accordingly, they have the ability to metabolize a multitude of structurally diverse compounds. It is likely that CYP3A enzymes are involved in the metabolism of more than 50% of all currently used drugs. There are 4 CYP3A genes, CYP3A4, CYP3A5, CYP3A7, and CYP3A43. CYP3A activities are thought to be the sum of the 3 isoenzymes, CYP3A4, CYP3A5, and CYP3A7. CYP3A43 is expressed at very low levels, and its relevance to drug disposition is not currently known.

CYP3A4 is considered the most abundant and clinically significant cytochrome P450 involved in drug metabolism. It is the major P450 isoform in the liver and gastrointestinal tract. During fetal stages, CYP3A7 is the predominant CYP3A expressed, but its expression declines after birth paralleled by an increase in CYP3A4 expression, which becomes the predominant isoform in adults. However, recent reports suggest that CYP3A7 is expressed at high levels in approximately 20% of adult livers. CYP3A5 is polymorphically expressed in the liver and intestine, and it is estimated that CYP3A5 isoenzyme is present in only 10% to 30% of livers. A study of livers of both Caucasian and African American origin found that CYP3A5 represented greater than 50% of the total CYP3A protein content in livers that expressed the isozyme, suggesting that CYP3A5 may play a significant role in the metabolism of CYP3A substrates. However, a recent study reported that CYP3A5 contributed only 17% of the total CYP3A protein in livers expressing CYP3A. In most drug biotransformations, CYP3A4 is a more efficient enzyme than CYP3A5, and because CYP3A4 expression levels seem to be higher than or similar to those of CYP3A5, the impact of CYP3A5 in adult liver drug metabolism is expected to be modest. On the other hand, CYP3A5 catalyzes the metabolism of several drugs more efficiently than CYP3A7.

An important characteristic of CYP3A is the large interindividual variation in the activity and levels of expression; however, the distribution of activity is continuous and unimodal, which suggests that multiple genes are involved in its regulation combined with modulation by environmental factors, but that individual genetic factors play a minor role. Several studies reported greater than 10-fold differences in the in vivo metabolism of CYP3A substrates and a 31-fold variation in the in vitro CYP3A4 activity. Such variation can cause clinically important differences in drug toxicity and response. Genetic factors appear to be of great importance for the interindividual variability in constitutive expression and activity of CYP3A enzymes accounting for 70% to 90% of the variation, although the underlying genetic factors are still not fully understood. A substantial number of allelic variants of CYP3A4 have been identified, but unlike other human P450s (CYP2D6, CYP2C19), there is no evidence of a “null” allele for CYP3A4. There have been 18 variant alleles described that are associated with nonsynonymous mutations. Although some of these amino acid substitutions give rise to altered catalytic function, they occur at low population frequencies, and most of the changes in catalytic activity observed for CYP3A4 gene variants are relatively modest. Thus, these coding variants may contribute to interindividual differences in CYP3A-dependent clearance but are not likely to be the major cause of the differences observed for the general population. The most common CYP3A4 variant that appears to have a modest functional consequence is the A-392G transition (CYP3A4*1B) in the 5’-flanking region. Although this allelic variant was reported to be associated with increased transcriptional activity in vitro, in vivo data are contradicting and suggest only a modest effect. In contrast, there are several reports linking this variant to various disease states including prostate cancer, secondary leukemias, and early puberty. Linkage disequilibrium between CYP3A4*1B and another CYP3A allele (CYP3A5*1) may be the true cause of the clinical phenotype.

Polymorphisms in the CYP3A5 have also been detected which explain variability in enzyme expression. The most frequent and functionally important SNP in the CYP3A5 gene consists of an A6986G transition within intron 3 (CYP3A5*3) that creates an aberrant splice site and results in a truncated non-functional protein. Only people with at least one wild-type CYP3A5*1 allele express large amounts of CYP3A5 isoenzyme, resulting in a 2.5-fold increase in clearance of the probe drug, midazolam. The CYP3A5*3 allele is common in all ethnic groups examined to date, however, there is marked ethnic variation in the frequency of this and other variant alleles. Among Caucasians, a CYP3A5*3/*3 genotype was perfectly concordant with very low or undetectable hepatic CYP3A5 protein content. The alleles CYP3A5*6 and CYP3A5*7 are more common in African Americans but are relatively rare among
CYP3A5*5 was first described in a Chinese population and is also associated with a splicing defect. Although there is considerable phenotypic variability within different CYP3A5 genotypes, livers carrying a functional CYP3A5 allele are more likely to exhibit high catalytic activity than those that do not. Because substrate specificity and product regioselectivity of CYP3A5 can differ from that of CYP3A4, it is likely that the impact of CYP3A5 genetic polymorphism on drug disposition will be drug dependent. Currently, there is no CYP3A5-selective substrate probe. However, there is still little information in the literature evaluating the in vivo significance of CYP3A5 pharmacogenetics with respect to metabolic drug clearance.

CYP3A5 is considered the primary, if not exclusive, extrahepatic CYP3A family member expressed in, for example, kidney, lung, prostate, breast, and polymorphonuclear leukocyte. It is interesting to note that salt-dependent renal hypertension is more prevalent in African Americans than in Caucasian Americans, as is the CYP3A5*1 allele. Polymorphic expression of CYP3A5 could also contribute to variable metabolism of steroids in the prostate and breast and to differences in the concentrations of circulating steroids and could be implicated in estrogen-mediated carcinogenicity.

The important role of CYP3A7 in the metabolism of endogenous substrates such as steroids and retinoic acid, in addition to xenobiotics reaching the fetus, has probably resulted in a well-conserved enzyme. CYP3A7*1C is associated with increased CYP3A7 expression in both liver and intestine. This allele was reported at a frequency of 3% in a Caucasian population and 6% in African Americans. The presence of CYP3A7*1B, a rarer allele, was associated with increased CYP3A7 expression only in the liver. Although no data are available regarding the functional significance of the CYP3A7*1C and CYP3A7*1B variants with regard to drug metabolism in vivo, one might anticipate increased metabolic clearance of its drug substrates based on observed polymorphic enzyme expression. Variable expression of CYP3A5 and CYP3A7 may account in part for the degree of variation seen in the metabolism of CYP3A4 substrates. However, it is still unclear whether this variable expression can explain the wide interindividual variation in CYP3A activity. Recent studies on polymorphism in PXR, a transcriptional regulator for CYP3A4, have identified several SNPs that may affect individual ability to induce CYP3A4. In addition, interindividual variation in levels of endogenous PXR ligands could explain some of the observed variability in CYP3A4 levels. More extensive research is needed to determine the ultimate impact of CYP3A genetic variation on human drug disposition, efficacy, and toxicity.

CONCLUSIONS AND FUTURE ASPECTS

Interindividual differences in the P450-mediated actions are caused both by environmental and genetic factors. Pharmacogenetics of the CYP P450 enzymes has helped to elucidate one aspect of the genetic basis for interindividual variability in drug response. However, polymorphisms in genes encoding drug transporters and receptors can also have a significant impact on the outcome of drug treatment. Genetic polymorphisms have now been identified in the genes encoding all the main cytochrome P450 enzymes that contribute to drug and other xenobiotic metabolism; nevertheless, a patient’s particular genotype is rarely determined in clinical practice. Lack of knowledge about genetics and pharmacogenetics among prescribers in addition to the lack of large, conclusive, prospective studies showing improvement of drug efficacy following genotyping are 2 major reasons for why incorporation of pharmacogenetic knowledge into routine medical practice is lagging. However, it is the application of this knowledge that will lead to achieving the ultimate promise of pharmacogenomics, which is personalized medicine, or optimum individualized treatments based on a patient’s genetic makeup.

Drugs that are ideal candidates for early evaluations of gene-based prescribing include those that are extensively metabolized by a polymorphic enzyme (eg, CYP2D6, CYP2C9, and CYP2C19), have a narrow therapeutic index, and those for which the number of therapeutic alternatives is limited. However, large prospective clinical trials are still needed to assess whether knowledge of a patient’s genotypic profile before drug prescribing increases drug efficacy, prevents or reduces adverse drug reactions, or lowers the overall cost of therapy. The costs of genotyping are decreasing rapidly, and our knowledge about the benefits of predictive genotyping for more effective therapy is increasing, indeed, higher throughput cost-effective genotyping techniques are becoming available. In December 2004, the FDA approved a microarray chip designed to routinely identify polymorphisms of drug-metabolizing enzymes related to cytochrome P450 drug metabolism. This technique, together with genotypes of transport proteins and drug receptors, will provide the physician with valuable information to individualize drug treatment.
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