9 Cytochrome P450 Polymorphisms

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9.1 SUMMARY

The majority of drugs in clinical use undergo oxidative biotransformations catalyzed by cytochrome P450 (CYP) enzymes. Approximately one dozen isoforms of the CYP families 1, 2, and 3 are mainly responsible for the metabolism of drugs and other xenobiotics. The typically large interindividual variability of these enzymes is an important determinant of drug pharmacokinetics and drug response. Both genetic and nongenetic factors contribute to this variability, but the degree to which genetic polymorphism plays a role in determining expression and function is different for each individual P450 gene. This chapter summarizes the enormous body of experimental evidence that has been gathered on the functional, clinical, and toxicological relevance of P450 genetic polymorphism. In the family CYP1, the *CYP1A1, CYP1A2*, and *CYP1B1* genes encode enzymes with preference for polycyclic and heterocyclic aromatic compounds. CYP1A2 is expressed at higher levels in liver, whereas CYPs 1A1 and 1B1 are mostly extrahepatic enzymes. While CYP1B1 mutations are causally involved in the development of congenital glaucoma, genetic polymorphism in the CYP1 family is of inferior significance for drug metabolism compared to nongenetic factors. In the largest human family CYP2, five subfamilies are of particular importance. Of the CYP2A genes *CYP2A6, CYP2A7*, and *CYP2A13*, the CYP2A6 polymorphism is an established determinant of nicotine metabolism with influence on addictive behavior. CYP2B6, the only functional enzyme encoded in the CYP2B subfamily, is highly polymorphic, and slow metabolizer genotypes are frequent among all major ethnic groups. The CYP2B6 polymorphism is important for HIV therapy with the antiretroviral drug efavirenz. The CYP2C subfamily comprises the four expressed genes *CYP2C8, CYP2C9, CYP2C18*, and *CYP2C19*. Despite their high homology, these genes differ tremendously with respect to expression, regulation, substrate selectivity, and genetic

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polymorphism. Clear genotype–phenotype correlations are established for CYP2C19 and CYP2C9. The historical polymorphism of *S*-mephenytoin hydroxylation, which is caused by loss-of-function alleles more prevalent among Asians than in other ethnic groups, recently gained importance for additional drugs. The polymorphism of CYP2C9 comprises amino acid variants that can have differential effects depending on the substrate and is of high clinical relevance. The strong genotype–phenotype correlation of CYP2D6 is in part due to the lack of gene regulation such as induction and on the existence of numerous single nucleotide polymorphisms (SNPs) and larger structural variations, including gene deletions, duplications, and recombinations. This results primarily in different expression levels leading to the ultrarapid metabolizer (UM), extensive metabolizer (EM), intermediate metabolizer (IM), and poor metabolizer (PM) phenotypes, the frequencies of which depend on ethnic background. The enzyme has a broad substrate selectivity comprising up to 20% of all drugs on the market. In contrast, CYP2E1, an ethanol-inducible P450, has a preference for low molecular weight compounds especially relevant for toxicological processes, which are only marginally influenced by genetic polymorphism. Often cited as the most important P450 subfamily, CYP3A comprises four functional genes, the most abundant and highly drug-inducible *CYP3A4* and the minor forms *CYP3A5, CYP3A7*, and *CYP3A43*. For *CYP3A4*, only few polymorphic markers of controversial value exist. *CYP3A5* and *CYP3A7* are polymorphically expressed but are of limited clinical importance because of their lower expression.

9.2 INTRODUCTION—SIGNIFICANCE OF P450 POLYMORPHISMS AND PHARMACOGENETICS

The human genome comprises 57 putatively functional, protein-coding CYP genes and 58 pseudogenes compared to 102 putatively functional genes and 88 pseudogenes in the mouse [1]. The human genes are grouped according to their sequence similarity into 18 families and 44 subfamilies (http://drnelson.utmem.edu/human.P450.table.html). The CYP1, 2, and 3 family isozymes have broad and overlapping substrate specificities, which usually provides for a robust elimination of lipophilic xenobiotics, including most drugs in clinical use [2–4]. In contrast, the CYPs of families CYP4 to CYP51 are mainly involved in endogenous metabolic pathways of steroids, fatty acids, prostaglandins, retinoids, and others [5]. The CYP1–CYP3 family isozymes show extremely variable expression and function, typically exceeding 100-fold within a given population sample, which leads to unforeseen drug responses including overreaction, toxicity, or lack of response in a considerable fraction of patients who have been treated with drug substrates of these enzymes. The reasons for this high interindividual and intraindividual variability include environmental influences such as drug–drug interactions, biological factors including sex and circadian rhythm, physiological determinants such as disease and hormonal status, and genetic polymorphisms in CYP genes and their regulators [3,4,6]. Genetic polymorphisms, although present in practically all human genes, affect only some CYP isoforms to a functionally relevant extent, including, in particular, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and the minor CYP3A forms CYP3A5 and CYP3A7. On the contrary, the highly abundant liver enzymes CYP1A2 and CYP3A4 are not functionally polymorphic because the known sequence variants only marginally affect their expression or function. The focus of this chapter is

to provide a summary of the medical relevance of the most important drug-metabolizing human CYPs of families CYP1, CYP2, and CYP3, with special emphasis on functionally relevant polymorphisms and their role in the metabolism of clinically relevant drugs. The numerous *in vitro* and *in vivo* pharmacogenetic studies that have been published on the subject illustrate the complex biology of these polymorphic genes at the level of basic mechanisms (*null* alleles, partially functional alleles, substrate-dependent effects, linkage disequilibrium, etc.) as well as their pharmacological, toxicological, and clinical relevance (PD (pharmacodynamics) vs PK (pharmacokinetics), adverse reactions, prodrugs, etc.). The website of the cypallele nomenclature committee served as the basis for naming variant alleles, and the reader is referred to this site for more detailed coverage of literature regarding individual allele variants ([7]; available at http://www.cypalleles.ki.se; consulted April 2010).

9.3 HUMAN DRUG-METABOLIZING CYTOCHROME P450s

9.3.1 Family *CYP1* —Overview

The human *CYP1A* family contains three functional genes in two subfamilies *CYP1A* and *CYP1B*. The highly conserved *CYP1A1* and *CYP1A2* genes, both of which consist of seven exons and six introns, are located on chromosome 15q24.1, whereas *CYP1B1* is located on chromosome 2p22.2 and contains three exons and two introns [1]. Many of the CYP1 substrates are ligands to the aromatic hydrocarbon receptor (AhR) that positively regulates the expression of *CYP1* and a battery of other genes in a coordinated manner at the transcriptional level [8,9]. The *CYP1A1* and *CYP1A2* genes are oriented head to head with a shared bidirectional promoter in between, which contains at least 13 AhR response elements [10]. Despite their common AhR-dependent regulation, the ontogenic patterns differ substantially, in that CYP1A1 is expressed more during embryogenesis, whereas CYP1A2 only appears around birth [11]. Pharmacologically most relevant is CYP1A2, which is constitutively expressed in liver, where it contributes on average $\sim$10–15% to the total microsomal P450 pool, but it is also expressed in intestine, pancreas, lung, and brain. CYP1A2 appears to display higher activity in men than in women [12,13]. Constitutive expression of CYP1A1 in liver is low, but inducible expression triggered by environmental inducers such as cigarette smoke, dioxin, and polycyclic hydrocarbons occurs ubiquitously in all tissues including lung and breast [14]. CYP1B1 is an extrahepatic enzyme expressed predominantly in various tumors and a useful tumor marker because of its absence in the corresponding normal tissues [15]. Notably, CYP1B1 is also expressed in various cell types of the human and mouse eye, where it has a yet undefined developmental role [16].

The substrates of the CYP1 enzymes include many polycyclic and other aromatic substances including diverse procarcinogens such as arylarenes, nitroarenes, and arylamines to reactive metabolites such as epoxide intermediates, which are carcinogenic in experimental animals and humans because they can cause DNA damage. Activation of the carcinogen benzo[*a*]pyrene is an important example of this type of metabolic activation [14]. CYP1A2 shows a preference for aromatic amines and heterocyclic compounds, including the two prototypic substrates theophylline and caffeine. Endogenous substrates are also known, for example, prostaglandins, estrogens, melatonin, and retinoic acid, some of which appear to affect the phenotype of $Ahr^{-/-}$ knockout mice

[17]. Only CYP1A2, because of its high expression in liver, metabolizes a significant number of clinically important drugs, including acetaminophen, phenacetin, lidocaine [18], and the CNS drugs olanzapine, clozapine, and duloxetine, among others [19,20]. Also of clinical importance is the fact that CYP1A2 is sensitive to drug interactions because of reversible and/or irreversible inhibition and induction by numerous drugs and several other xenobiotics. The structure of human CYP1A2 in complex with the inhibitor α-naphthoflavone was recently presented at a resolution of 1.95 Å, revealing a compact active site cavity highly adapted for the positioning and oxidation of relatively large, planar substrates [21]. CYP1B1 has catalytic activities overlapping with CYP1A1 and CYP1A2 and also catalyzes activation of diverse procarcinogens, but the catalytic role depends on the substrate and the expression level in each tissue [22].

9.3.1.1 Subfamily CYP1A—CYP1A1 and CYP1A2.

9.3.1.1.1 CYP1A1. The *CYPallele* website lists 10 variant alleles and a number of subvariants, as well as additional SNPs, where the haplotype has not yet been determined. Four early identified, more common variants were originally termed m1–m4. The m1 variant (*CYP1A1*2A*), which harbors a 3698 T>C transition creating a new MspI site downstream of the polyadenylation site, and the m2 variant (*CYP1A1*2C*) with the key mutation Ile462Val near the heme-binding region were associated with greater enzymatic activity in some but not all studies [14,23]. These variants are common among Asians (∼35% and 20%, respectively, Table 9.1) and have been proposed as genetic susceptibility factors for lung cancer in the Japanese [24,25], but this finding was not confirmed in other populations, possibly because of the much lower allele frequencies [26]. The m3 variant (*CYP1A1*3* creating an Msp1 site in the 3′ noncoding region) appears to be an African-specific mutation that does not occur in the other large ethnicities. The m4 variant is located near the m2 mutation site and codes for an amino acid change (Thr461Asn) near the heme-binding region. Its frequency is quite low (∼3%) in Caucasians, and no association with lung cancer was found [26]. Besides these variants, other coding polymorphisms (in total to 16 amino acid variants) were described but functional data is scarce.

Because many CYP1A substrates are procarcinogens occurring in industrial combustion products, cigarette smoke, and charred food, polymorphisms in the human *CYP1A* family genes have been investigated mostly as risk factors for various forms of cancer including breast and lung cancer. The evidence for association of CYP1A1 polymorphisms with cancer is controversial, as some studies reported increased susceptibility, whereas others have reported no relationship, although most experimental data support positive associations for the more common variants [27–33]. Further investigation is required with larger clinical studies, consideration of environmental and other nongenetic factors, more detailed haplotype analysis, as well as a more rigorous functional assessment of the variants.

9.3.1.1.2 CYP1A2. Like other drug-metabolizing enzymes, CYP1A2 expression in human liver is highly variable between individuals, which is certainly due to several factors including genetic, epigenetic, and environmental factors (e.g., smoking [34]). By measuring the caffeine metabolic ratio as a CYP1A2 activity marker in a large cohort ($n = 378$) of mono- and dizygotic twins selected to exclude the influence of smoking, oral contraceptives, and gender, Brøsen and colleagues found a strong overall heritability of 0.72 [35]. To date, more than 15 variant alleles and numerous haplotype variants

TABLE 9.1 Genetic Polymorphisms of Human cytochrome P450s with Functional Significance

CYP Allele Designation[a]	Key Mutation(s)[b]; rs Number	Location; Protein Effect	Allele Frequencies[c]	Functional Effect
*CYP1A1*2A*	3698T>C; rs4646903	3′ untranslated region; MspI site created	0.05–0.07 Ca ~0.30–0.40 As 0.82 In 0.04–0.28 SA	Unclear
*CYP1A1*2C*	2454A>G; rs1048943	I462V	0.03 Ca ~0.20 As 0.82 In 0.09–0.9 SA	Unclear
*CYP1A2*1C*	−3860G>A; rs2069514	Promoter	0.26–40 AA, Af 0.01–0.08 Ca 0.21–0.27 As 0.20–0.30 Hs 0.24 Pc	↓ Inducibility (smokers)
*CYP1A2*1F*	−163C>A; rs762551	Intron 1	0.5–0.8 all ethnicities	↑ Inducibility (smokers, omeprazole)
*CYP1B1*6*	142C>G; rs10012 355G>T; rs1056827 4326C>G; rs1056836	R48G A119S L432V	0.5 Af 0.4 Ca 0.12 As	↑ K_m, ↓ V_{max} (17β-estradiol, recombinant)
*CYP1B1*7*	*6 + 4360C>G	A443G	0.07 Af 0.026 Ca	↑ K_m, ↓ V_{max} (17β-estradiol, recombinant)
*CYP2A6*2*	1799T>A; rs56844942	L160H	0.01–0.05 Ca 0.01 AA 0.00 As	No activity
*CYP2A6*4A/D*	Recombination	CYP2A6 deleted	0.01–0.04 Ca 0.01–0.02 AA 0.05–0.24 As	No activity

(*continued overleaf*)

TABLE 9.1 (*continued*)

CYP Allele Designation[a]	Key Mutation(s)[b]; rs Number	Location; Protein Effect	Allele Frequencies[c]	Functional Effect
*CYP2A6*7*	6558T>C; rs5 031 016	I471T	0.00 Ca 0.00 AA 0.06–0.13 As	Decreased activity
*CYP2A6*9*	−48T>G; rs28 399 433	TATA box	0.05–0.08 Ca 0.08 AA 0.16–0.20 As	Decreased activity
*CYP2A6*17*	5065G>A; rs28 399 454	V365M	0.00 Ca 0.10 AA 0.00 As	Decreased activity
*CYP2B6*4*	18053A>G; rs2 279 343	K262R	0.00 AA, Af 0.04 Ca 0.07 As	↑ Expression and activity
*CYP2B6*6*	15631G>T; rs3 745 274 18053A>G; rs2 279 343	Q172H K262R	0.33–0.5 AA, Af 0.17 As 0.25 Ca 0.37 Hs	↓ Expression and activity
*CYP2B6*18*	21011T>C; rs28 399 499	I328T	0.04–0.07 AA, Af 0.00 As, Ca, Hs	↓ Expression and activity
*CYP2C8*2*	1 1054A>T; rs11 572 103	I269F	0.10–0.21 AA, Af 0.00 As, Ca	↓ Activity
*CYP2C8*3*	2130G>A; rs11 572 080 3 0411A>G; rs10 509 681	R139K K399R	0.13 Ca 0.00 AA, Af, As	↓ Activity (paclitaxel) ↑ Activity (antidiabetics)
*CYP2C9*2*	3608 C>T; rs1 799 853	R144C	0.07 AA, Hs 0.00 Af 0.13–0.17 Ca 0.02 Pc	↓ Activity

*CYP2C9*3*	4 2614A>C; rs1 057 910	I359L	0.00–0.01 Af, AA 0.04 As 0.06 Ca	↓↓ Activity
*CYP2C19*2*	19154G>A; rs4 244 285	Splicing defect	0.10–0.16 AA, Af 0.22–0.32 As 0.15 Ca	*Null* allele
*CYP2C19*3*	17948G>A; rs4 986 893	W212X	0.00–0.01 Af, Ca 0.03–0.07 As, Pc	*Null* allele
*CYP2C19*17*	−806C>T; rs12 248 560	Promoter	0.15–0.25 AA, Af, Ca 0.04 As	↑ Expression and activity
*CYP2D6*3*	2549delA; rs35 742 686	Frameshift	0.00–0.01 all ethnicities	*Null* allele
*CYP2D6*4*	1846G>A; rs3 892 097	Splicing defect	0.15–0.25 Ca <0.01 Most others	*Null* allele
*CYP2D6*5*	Recombination	Deletion	0.03–0.06 All ethnicities	*Null* allele
*CYP2D6*6*	1707delT; rs5 030 655	Frameshift	0.00–0.01 All ethnicities	*Null* allele
*CYP2D6*10*	100 C>T; rs1 065 852	P34S	0.02 Ca 0.40-0.50 As	↓ Expression and activity
*CYP2D6*17*	1023C>T; rs28 371 706 2850C>T; rs16 947	T107I R296C	0.34 Af 0.00 As, Ca	↓ Expression and activity
*CYP2D6*41*	2988G>A; rs28 371 725	Splicing defect	0.085 Ca < 0.01 All others	↓ Expression and activity
*CYP2D6*Nxn*	Recombination	Copy number variations	0.01–0.09 Ca up to 0.30 Af, Ar	↑ Expression and activity
*CYP3A4*1B*	−392A>G; rs2 740 574	Promoter	0.68–0.82 AA, Af 0.00 As 0.03–0.05 Ca, Hs	Probably effect on transcription

(*continued overleaf*)

TABLE 9.1 (*continued*)

CYP Allele Designation[a]	Key Mutation(s)[b]; rs Number	Location; Protein Effect	Allele Frequencies[c]	Functional Effect
*CYP3A5*3*	6986A>G; rs776 746	Splicing defect	0.37 AA 0.12–0.15 Af 0.66–0.75 As, Hs 0.94 Ca	↓ Expression and activity
*CYP3A7*1C*	Several SNPs	Functional ER6 element created in the promoter region	0.06 AA 0.04 Ca 0.00 As	Increased expression

[a] According to the CYPallele nomenclature homepage (http://www.cypalleles.ki.se).
[b] Genomic positions are given.
[c] Frequencies of the indicated variant alleles were obtained for different ethnicities (AA, African-American; Af African; As, Asian; Ar, Arab; Ca, Caucasian; Hs, Hispanic; In, Indian; Pc, Pacific; SA, South American) from dbSNP at http://www.ncbi.nlm.nih.gov/SNP, from the Allele Frequency Database (ALFRED) at http://alfred.med.yale.edu/alfred/index.asp, and from the references indicated in the text.

of the *CYP1A2* gene have been defined, and some of them have been associated with altered drug clearance and response and with disease susceptibility. However, it appears that the basis for inheritable CYP1A2-dependent phenotypes has not been satisfactorily elucidated. In a recent study, the human *CYP1A1-CYP1A2* locus was resequenced in 5 major ethnicities phenotyped, revealing a total of 85 SNPs, 57 of which occurred more than once [36]. Attempts to relate the most common of these SNPs to phenotype in genotyped volunteers were disappointing, and it was concluded that no SNP or haplotype in the *CYP1A2* gene has a clear predictive value [37]. Among the polymorphic sites in the coding and noncoding regions in the *CYP1A2* gene, only few common SNPs are currently considered to be of potential predictive value, including the 5′-upstream variant −3860G>A (*CYP1A2*1C*) and the intron 1 polymorphism −163C>A (*CYP1A2*1F*) located downstream of the untranslated first exon. Functional evidence for the *CYP1A2*1C* variant suggests decreased inducibility by smoking on the basis of promoter analyses and its association with decreased caffeine 3-demethylation in Japanese smokers [38]. In contrast to the **1C* variant, the intron 1 variant, *CYP1A2*1F*, was associated with increased enzyme inducibility in German [39] and Swedish [34] smokers and with "ultrarapid" caffeine metabolism and nonresponse to clozapine in smoking patients with schizophrenia [40]. The opposite effect of the two variants is consistent with the findings that carriers of the combined genotype *CYP1A2*1C/*1F* were not induced by omeprazole [41]. The *CYP1A2* genotype also modifies the risk of hypertension in coffee drinkers, who were at increased risk when they carried the slow **1F* allele [42]. Although slower metabolism of caffeine was confirmed in Chinese breast cancer patients, the variant was not related to disease risk [43]. However, some data with different substrates are contradictory, as no influence was found for clozapine [44], haloperidol [45], and trazodone [46]. These discrepancies may be due to ethnically diverse haplotype patterns.

CYP1A2 polymorphisms that lead to amino acid changes are generally rare, but for some of them, decreased activity could be shown in recombinant expression systems. The Arg431Trp substitution (*CYP1A2*6*), which is located in the "meander" peptide, a region critical for maintenance of protein tertiary structure, was shown to interfere with binding and to result in nonfunctional protein [47]. However, because of their rare occurrence, this and other amino acid variants [7] are of limited clinical use. In Chinese subjects phenotyped with caffeine, a common haplotype containing the novel SNP −3113G>A was associated with significantly lower metabolic activity. This SNP is located in a proposed regulatory element, but it remained unclear whether it is causally responsible for the effect [48].

In conclusion, the currently known polymorphisms in the *CYP1A2* gene explain only a small fraction of the CYP1A2 expression and functional variability. Although novel SNPs may still be discovered, genetic determinants in other genes that contribute to the regulation of CYP1A2 expression, including the AhR pathway, inflammation signaling pathway, or other signaling pathways, may be involved. Although AhR polymorphisms were reported [49,50], systematic investigations and an evaluation of their effects on CYP1A2 phenotypes are scarce.

9.3.1.2 Subfamily CYP1B—CYP1B1. CYP1B1 is the first CYP that was shown to be involved in a primary developmental defect if mutated. It was identified by genetic linkage analysis and mutation studies as a causative gene in primary congenital glaucoma, an inheritable neurodegenerative disease leading to blindness [51].

Because CYP1B1 may be involved in the metabolism of steroids, arachidonic acid, vitamin A, and melatonin, the enzyme is believed to be responsible for the generation of an as yet undefined signaling molecule(s) that is somehow involved in the development and pathogenesis of glaucoma. Over 80 mutations have been identified in patients with various forms of glaucoma [51]. Most of them are missense or nonsense mutations, or deletions, insertions, and/or duplications. Some of them are predicted to interrupt the open reading frame, and some were shown to lead to severely compromised enzyme function [51–54]. Because these mutations are rare in the general population, their value as tumor markers or markers of altered metabolism of drugs may be limited.

At least six more common polymorphic alleles, which contain five amino acid changes in different combinations, also exist in the *CYP1B1* gene. Functional analysis of four variant proteins coexpressed with P450 reductase in *Escherichia coli* revealed little influence of the Arg48Gly, Ala119Ser, and Asn453Ser variants on kinetic properties toward 17β-estradiol as substrate, but a threefold increase in K_m for the Leu432Val (*CYP1B1*3*) variant [55]. An expression study in yeast found contradictory data regarding the Leu432Val variant, in that no change was seen for the individual variant using the same substrate but decreased V_{max} and increased K_m values toward 17β-estradiol when additional amino acid changes were present in the CYP1B1.6 and CYP1B1.7 alleles [56]. The CYP1B1.7 variant was also shown to have decreased capacity to hydroxylate benzo[*a*]pyrene. The Leu432Val variation was also correlated to changed urinary estrogen metabolites, indicating *in vivo* contribution of CYP1B1 to catabolism of estrogen [57]. These functional *in vitro* studies suggest that these common amino acid changes in the *CYP2B6* gene lead to little or moderate but potentially substrate-dependent changes in the catalytic properties of the enzyme.

Association of *CYP1B1* genotype with various forms of cancer was reported including a possible effect on breast cancer risk in Caucasians but not in Asians [58]. Regarding three further *CYP1B1* polymorphisms resulting in amino acid changes Arg48Gly, Ala119Ser, and Asn453Ser, a recent meta-analysis concluded that these polymorphisms are not associated with breast cancer risk [59]. In a large study that included more than 10,000 patients, *CYP1B1* variants **3* [Leu432Val] and **4* [Asn453Ser], which influence the metabolism of polycyclic aromatic hydrocarbons of tobacco smoke, were also not associated with the risk of various diseases including chronic obstructive pulmonary disease and tobacco-related lung cancer [60].

9.3.2 Family *CYP2* —Overview

The *CYP2* family is the largest family in humans. It contains 16 full-length genes and all have 9 exons and 8 introns. Among them are most of the important drug-metabolizing CYPs expressed in liver as well as several extrahepatic enzymes, for some of which the function has yet to be elucidated ("orphan" CYPs [61]). The genes are organized in a number of multigene clusters that can contain one or more subfamilies and are spread over different chromosomes. The three largest gene clusters are the *CYP2ABFGST* cluster on chromosome 19q13.2, the *CYP2C* cluster on chromosome 10q23.33, and the *CYP2D* cluster on chromosome 22q13.1–2. In the evolution of rodents, many of the *CYP2* subfamilies expanded tremendously, making direct comparisons between mouse and human P450s especially challenging in this family [1].

Almost all *CYP2* genes are highly polymorphic. This chapter describes those genes that are of highest importance for xenobiotic metabolism.

9.3.2.1 Subfamily CYP2A—CYP2A6, CYP2A7, and CYP2A13.

9.3.2.1.1 Molecular Genetics and Pharmacological Properties. The human *CYP2A* genes are part of a 350-kb *CYP2ABFGST* gene cluster on chromosome 19q13.2 that contains genes and pseudogenes of the *CYP2A, 2B, 2F, 2G, 2S*, and *2T* subfamilies [1]. It contains the four *CYP2A* members *CYP2A6, CYP2A7, CYP2A13*, and a split pseudogene *CYP2A18P*. In humans, CYP2A6 is mainly expressed in the liver where it represents on average about 2% of the total liver P450 protein, whereas in extrahepatic tissues, it was found only in trace amounts. CYP2A7 is a nonfunctional enzyme that is unable to incorporate and exhibit catalytic activity in various heterologous systems [62]. *CYP2A13* codes for a catalytically active protein and is predominantly expressed in the respiratory tract, where expression levels decrease from nasal mucosa to peripheral lung tissues [63,64].

Human CYP2A6 has been recognized as the major isoform involved in the oxidative metabolism of the psychoactive tobacco ingredient nicotine to the inactive cotinine [65,66]. A rather specific marker activity for CYP2A6 is the 7-hydroxylation of coumarin [67,68]. Up to 300-fold interindividual differences in CYP2A6-mediated coumarin 7-hydroxylase activity were observed, which is influenced by ethnicity, as only 1% of white subjects are PMs compared to up to 20% of Asians [69,70]. CYP2A6 is also the main isoenzyme responsible for the minor 7-hydroxylation of efavirenz [71]. Furthermore, CYP2A6 contributes to the metabolism of a number of clinically used drugs such as disulfiram, fadrozole, halothane, letrozole, losigamone, methoxyflurane, and valproic acid [72]. CYP2A13 has similar substrate specificity and also metabolizes coumarin and nicotine, but it has been shown to be the most efficient metabolic activator of nicotine-derived nitrosamine ketone (NNK), a major tobacco procarcinogen [73].

9.3.2.1.2 Genetic Polymorphisms and Pharmacogenetics. Genetic variation in the *CYP2A6* gene can both increase or decrease enzyme activity because it includes alleles with deleted or duplicated genes, gene conversions, nucleotide deletions and insertions, as well as numerous coding and noncoding SNPs. These variants may change mRNA and/or protein expression levels or affect the structure and function of the protein [74,75]. The CYPallele website currently lists 37 distinct alleles, that is, variants that cause amino acid changes or with proven functional effect. Many of these variants occur in various combinations, giving rise to complex haplotypes that occur at distinct ethnic frequencies [76]. A detailed compilation of the known structural changes and their functional impact was presented by Mwenifumbo and Tyndale [74]. The prevalence of functionally relevant *CYP2A6* alleles was recently estimated to be ~9% in Caucasians, ~22% in Africans, and up to 50% in Asians [77].

The *CYP2A6*2* [Leu160His] and the **4A-H* deletion alleles have been shown to dramatically reduce enzyme activity *in vivo* in homozygous or hemizygous combination, resulting in the PM phenotype in affected individuals (Table 9.1). The most frequent of these presumable *null* alleles in Asians are the **4A-H* hybrid-deleted alleles that consist of a *CYP2A7*-derived 5′ part and a 3′ part of *CYP2A6* origin. The **4A* deletion variant is rare in Caucasians and Africans but present with up to ~20% frequency in Asian populations [76]. The *CYP2A6*2* [Leu160His] codes for an unstable

protein that fails to incorporate heme, but its frequency is only ~1% to 5% in Caucasians and is lower in Africans and Asians [76]. In a study involving 156 liver samples from Caucasians, resequencing revealed 33 haplotypes of which two (**9B*, containing a −48T>G TATA-box polymorphism, and the 2A7/2A6 recombination allele **12B*) were major genetic determinants associated with decreased hepatic expression [78].

A number of additional alleles including *CYP2A6*7, *10,* and **17* severely reduce enzyme activity in homozygous or hemizygous individuals. The **7* [Ile471Thr] and **10* [Ile471Thr; Arg485Leu] alleles are Asian specific, and **17* [Val365Met] was only found in African-Americans. The *CYP2A6*5, *6, *11,*19,* and **20* alleles were shown to have reduced activity by heterologous expression, but their low frequencies did, so far, not allow to confirm this *in vivo*. A further number of variants were shown to affect expression or function less dramatically, including several promoter variants that result in decreased expression, and some variants have not been characterized yet [74]. The *CYP2A6* gene was found in a duplicated variant in at least two different allele variants (**1X2A* and **1X2B*), which appear to be correlated to increased activity [79,80]. A decreased gene copy number is associated with gene deletions of the CYP2A6*4A–4H alleles.

Most pharmacogenetic studies involving *CYP2A6* were carried out to study the effect of genotype on nicotine metabolism, smoking behavior, or lung cancer risk.

An *in vivo* study in 278 healthy Caucasian volunteers investigated the relationship between variant CYP2A6 genotypes and the disposition and metabolism of intravenously administered nicotine [81]. Genotype-dependent differences in nicotine and cotinine plasma clearance were observed with the group, including the normal activity alleles having the largest capacity for nicotine C-oxidation. In a nicotine-replacement clinical trial, the group with slow metabolizer alleles had 50% reduced CYP2A6 activity before treatment and reached 44% higher steady-state plasma levels of nicotine [82]. A large interethnic variability study compared nicotine metabolism and CYP2A6 genotype in white, black, Korean, and Japanese subjects [76]. Large interindividual differences were observed in the cotinine/nicotine ratios in plasma. While no difference was found in the metabolic ratio of white, black, and Korean subjects, Japanese subjects showed a significantly lower metabolic ratio. The influence of low activity alleles on nicotine metabolism was confirmed in this study.

In contrast to the influence on pharmacokinetics, association of genetically variant *CYP2A6* with smoking behavior or lung cancer risk is still debated. The reader is referred to recent specialized reviews on these topics [74,75,83].

CYP2A6 polymorphism also plays a role for other substrates. A recent study emphasized the role of CYP2A6 for efavirenz, an antiretroviral drug usually metabolized by CYP2B6 to the 8-hydroxyderivative. As initially shown in human liver microsomes, the minor 7-hydroxy-efavirenz metabolite is mainly formed by CYP2A6 [71]. The wide interindividual variability in efavirenz plasma exposure is largely explained by CYP2B6 polymorphisms (see below). However, in patients with genetically impaired CYP2B6, substrate flux via the CYP2A6-dependent pathway is increased. The accessory metabolic pathway was shown to be critical in limiting drug accumulation in individuals characterized as CYP2B6 slow metabolizers [77]. As combined CYP2B6 and CYP2A6 genetic deficiency occurs at significant frequency in various human populations, the CYP2A6 polymorphism may be a clinically relevant determinant of extremely high efavirenz exposure in HIV patients.

9.3.2.2 Subfamily CYP2B—CYP2B6.

9.3.2.2.1 Molecular Genetics and Pharmacological Properties. The human *CYP2B* subfamily consists of the functional *CYP2B6* gene and the nonfunctional pseudogene *CYP2B7P*, which are located in a tandem head-to-tail arrangement within the large *CYP2ABFGST* gene cluster on chromosome 19 [1]. Expression of human CYP2B6 was initially underestimated [84], but despite higher estimations of more recent studies, it is one of the weaker expressed P450s, contributing on average ~2% to the total hepatic P450 pool [85]. CYP2B6 is also detectably expressed in human brain, kidney, heart, intestine, uterine endometrium, skin, and various tissues of the respiratory tract, including lung and nasal mucosa [64,86,87].

The CYP2B6 enzyme metabolizes diverse chemicals including several clinically used drugs as well as a large number of environmental chemicals. Therapeutically important drug substrates include the prodrug cyclophosphamide, which is activated by CYP2B6 to the cytotoxic 4-hydroxy-metabolite; the antiretroviral drugs efavirenz and nevirapine; the antidepressant and smoking cessation agent bupropion; the benzodiazepine diazepam; the antimalarial artemisinin; the anesthetics propofol and ketamine; and the synthetic opioid methadone [88–90]. Several suitable probe drugs for CYP2B6 have been discussed, but bupropion hydroxylation appears to be the most selective one [91] and is now most often used. However, because bupropion hydroxylation is not a sensitive marker for pharmacogenetic differences *in vivo* [92], other biotransformations may be more suitable markers. Liver microsomal efavirenz 8-hydroxylation has been shown to be correlated to CYP2B6 protein amount and is highly dependent on genotype *in vivo* (see below). Its usefulness as a suitable probe drug for CYP2B6 has, however, not yet been formally shown. CYP2B6 also metabolizes the N-demethylation of the recreational drug ecstasy (MDMA), which leads to potentially neurotoxic metabolites [93]. Nondrug xenobiotics metabolized mainly by CYP2B6 include the organophosphorus insecticide chlorpyrifos, which is metabolized to the more toxic oxon metabolite; the insecticide and endocrine disruptor methoxychlor; and the extensively used insect repellent DEET (*N,N*-diethyl-*meta*-toluamide) (for review, see Ref. 94).

Hepatic CYP2B6 is highly variable. In our study of 235 human liver samples, CYP2B6 protein varied ~360-fold, and all samples expressed at least a very low amount of the protein [85,95]. In this and other studies [96], no significant sex difference in CYP2B6 expression was found, but the issue remains controversial as other studies reported gender differences with higher expression in females versus males, which were more pronounced among Hispanics than Caucasians [97]. Several other mechanisms have been shown to contribute to inter- and intraindividual variability in hepatic CYP2B6 function, including induction of transcription by xenobiotics, [98] reversible and irreversible inhibition by diverse substances, [89] and genetic polymorphism [95]. As the human ortholog to the rodent phenobarbital-inducible CYP2b9/2b10 enzymes, CYP2B6 induction by drugs and other xenobiotics is due to similar mechanisms involving the xenosensors constitutive androstane receptor (CAR) and/or the pregnane X receptor (PXR) [98–100]. Known inducers are, for example, rifampin, barbiturates, cyclophosphamide, artemisinin, carbamazepine, efavirenz, and nevirapine, as well as metamizole [99,101] and statins [102]. Although this environmentally triggered variability should be expected to mask pharmacogenetic phenotypes, *CYP2B6* polymorphisms turned out to be highly significant for pharmacokinetics and possibly response to certain drugs, in particular, the anti-HIV drug efavirenz.

9.3.2.2.2 Genetic Polymorphisms and Pharmacogenetics. The CYPallele website currently lists 29 distinct alleles, that is, variants that cause amino acid changes or with a proven functional effect. More than 30 SNPs code for amino acid changes, which occur in numerous complex haplotypes and with distinct ethnic frequencies [90]. The most common variant *CYP2B6*6* harbors two amino acid changes Gln172His and Lys262Arg, which occur in various combinations with other changes that are apparently of minor functional significance. The **6* allele occurs with frequencies between 15% and 60% across different populations (Table 9.1), and it is associated with 50–75% decreased hepatic protein expression [71,95,97]. The causal variant for decreased expression was identified as the c.516G>T [Gln172His] polymorphism, which prevents correct splicing of the CYP2B6 pre-mRNA and leads to a shorter mRNA that lacks exons 4–6 [85]. The second most important functionally deficient allele is *CYP2B6*18* (c.983 C>T [Ile328Thr]), which did not form a functional protein in transfected mammalian cells and may thus be a *null* allele [103]. This allele was not found in Caucasians. In African populations, it occurs with a frequency of up to 7% (Table 9.1). Most other functional polymorphisms occur at lower frequencies [95,103–106]. An allele associated with increased transcription *in vitro* was also identified (*CYP2B6*22*, [105]). The −82T>C change was shown to alter the TATA box into a functional CCAAT/enhancer-binding protein binding site that causes increased transcription at an alternative downstream initiation site [105].

The major clinical role of *CYP2B6* polymorphism was elucidated after the *in vitro* studies identified CYP2B6 as the enzyme responsible for the conversion of efavirenz into its major 8-hydroxylated and 8-, 14-dihydroxylated metabolites [107]. Efavirenz is a potent agent recommended for initial therapy in regimens with two nucleoside reverse transcriptase inhibitors (NRTIs), but patients with subtherapeutic plasma concentrations can develop resistance and treatment failure, whereas those with too high plasma concentrations are at increased risk of CNS side effects. Several clinical studies found that HIV-infected patients homozygous for the 516 T allele develop several-fold higher median AUC values compared to those with only one or no T-allele [108]. The 516 T variant was also associated with increased CNS side effects [109] and treatment discontinuation [110]. In a prospective, genotype-based dose adjustment study, the therapeutic dose of efavirenz could be successfully reduced and CNS-related side effects decreased [111]. Furthermore, it was shown that analysis of rare loss-of-function alleles can improve prediction of high efavirenz plasma levels [106].

CYP2B6 genotype also affects plasma levels of the antiretroviral drug nevirapine [112]. However, the common *CYP2B6* polymorphisms appear to be less penetrant for some other substrates, probably because the amino acid changes can cause additional effects. A single-dose pharmacokinetic study in volunteers revealed no change in total bupropion clearance, although C_{max}, AUC, and metabolic ratio for hydroxybupropion were lower in **6/*6* and **1/*6* compared to **1/*1* [92]. In this study, a higher clearance was found for allele **4*, consistent with higher activity of the K262R variant in an *in vitro* system [113]. Cyclophosphamide, one of the most widely used anticancer and immunosuppressant drug, requires bioactivation by P450 enzymes to yield the active 4-hydroxy-metabolite, and CYP2B6 was shown to be the major enzyme for this step [114]. Studies reported that cyclophosphamide bioactivation may be enhanced by the c.516G>T allele in white subjects, further emphasizing the possibility of substrate-dependent effects of these amino acid variants [115]. CYP2B6 allele variants were also investigated in the context of the synthetic μ-opioid receptor agonist, methadone,

which is used as a maintenance treatment for opioid addiction, and in **6/*6* carriers, (*S*)-methadone plasma levels were increased, leading to potentially higher risk of severe cardiac arrhythmias and sudden death [116,117].

In conclusion, the clinical impact of *CYP2B6* pharmacogenetics has only recently been investigated. *CYP2B6* genotyping predicts elevated plasma concentrations of efavirenz and nevirapine in HIV-infected individuals. This is in agreement with basic *in vitro* studies showing lower expression as a consequence of erroneous splicing of the most common **6* variant. Substrate-dependent effects have to be expected because of the presence of amino acid changes in the polymorphic alleles.

9.3.2.3 Subfamily CYP2C—CYP2C8, CYP2C9, CYP2C18, and CYP2C19.

9.3.2.3.1 Molecular Genetics and Pharmacological Properties. The human *CYP2C* subfamily consists of the four genes *CYP2C18, 2 C19, 2 C9*, and *2 C8*, which are localized in this order in a gene cluster on chromosome 10q24. Although CYP2C18 mRNA is highly expressed in liver, the transcript is not efficiently translated into protein and, therefore, does not make significant contributions to drug metabolism. The other three *CYP2C* members together constitute on average between 30% and 50% of the microsomal hepatic P450 pool, with CYP2C8 and CYP2C9 being expressed roughly 10-fold higher than CYP2C19. At lower levels, functional CYP2C enzymes were also detected [63], for example, in human small intestine, where they are independently regulated, and in cardiovascular tissues [118]. All three expressed CYP2C enzymes are inducible by ligands of the PXR/CAR and glucocorticoid (GR) nuclear receptor pathways through different response elements in their upstream regulatory regions [119]. However, the relative inducibilites are different for the three genes. All four *CYP2C* genes are genetically polymorphic. The CYP2C19 or "*S*-mephenytoin" polymorphism is one of the early examples of P450 polymorphisms [120]. *CYP2C19* null alleles are common enough among Caucasians and Asians to produce a homozygous PM phenotype. In contrast, the common CYP2C8 and CYP2C9 variants code for full-length proteins with amino acid changes that lead to more moderate and substrate-dependent functional changes.

9.3.2.3.2 CYP2C8. CYP2C8 is mainly responsible for the metabolism of the antidiabetics rosiglitazone and pioglitazone, the antiarrhythmic amiodarone, as well as the retinoic acid drugs used in acne and cancer treatment. Some overlap in substrate specificity with CYP2C9 occurs, for example, as in the case of ibuprofen [4,121]. Additional important drug oxidations catalyzed primarily by CYP2C8 are the 6α-hydroxylation of the natural anticancer drug paclitaxel and the deethylation of the antimalarial amodiaquine, both useful probe drugs for CYP2C8 phenotyping. In addition to amodiaquine, CYP2C8 has a major role in metabolizing other antimalarials such as chloroquine and dapsone [122]. The clinical significance of CYP2C8 became apparent after its involvement in fatal drug interactions was described, which were in part due to its potent inhibition by gemfibrozil acyl-glucuronide, ultimately leading to cerivastatin-induced rhabdomyolysis [123,124]. Many more potent inhibitors at clinically relevant concentrations have been described [125].

Apart from rare variants with no (**5, *7*), reduced (**8*), or unknown activity, there are three more common alleles *CYP2C8*2, *3,* and **4*, which code for amino acid variants (Table 9.1). The **2* allele [Ile269Phe] most frequently (15–20%) occurs in individuals of African origin, and much lower to no occurrence is observed in

Caucasians and Asians [126–128]. The heterologously expressed variant had twofold increased intrinsic clearance for paclitaxel because of increased K_m. The **3* allele [Arg139Lys + Lys399Arg] occurs more frequently in white subjects but is almost absent in Africans and Asians. The CYP2C8.3 variant was initially shown to reduce metabolism of arachidonic acid and paclitaxel hydroxylation *in vitro* by ~40% and ~80%, respectively [126]. The evidence for altered pharmacokinetics, toxicity, or efficacy of these drugs in carriers of any of these alleles is however controversial. Owing to the close distance, the *CYP2C8*3* variant is in partial linkage disequilibrium to the *CYP2C9*2* allele, which may be of particular importance for substrates metabolized by both enzymes such as ibuprofen. Indeed, *CYP2C8*3* genotypes were associated, together with *CYP2C9*2* and **3* alleles, with reduced clearance of ibuprofen [129]. Some functional effects of *CYP2C8*3* are substrate dependent, for example, for repaglinide and rosiglitazone, higher metabolic capacity of this variant was observed. The functionally less-well-investigated **4* allele occurs in Caucasians (~8%) and is practically absent from all other major races. A recent review on CYP2C8 pharmacogenetic studies can be found in Ref. 130.

In conclusion, the most relevant *CYP2C8* variants are the alleles *CYP2C8*2* and *CYP2C8 *3* that code for amino acid variants whose frequencies vary between 7.5% and 20% depending on ethnicity. Their functional effects appear to be substrate dependent.

9.3.2.3.3 CYP2C9. In liver, CYP2C9 is one of the most abundantly expressed P450s. It accepts weakly acidic substances such as the anticoagulant warfarin, the anticonvulsants phenytoin and valproic acid, cardiovascular drugs such as rosuvastatin and losartan, and several nonsteroidal anti-inflammatory drugs (NSAIDs, [131]). Many of these drugs have a narrow therapeutic index, and variations in CYP2C9 activity are thus among the recognized factors for adverse drug reactions. The CYP2C9 enzyme also metabolizes endogenous substances, in particular, arachidonic acid and some steroids, and the fact that it is expressed in endothelial cells implicates it in the regulation of vascular tone [132].

Among the 34 distinct alleles listed on the CYPallele website, the initially discovered alleles, **2* [Arg144Cys] and **3* [Ile359Leu], have been investigated more thoroughly than the more recently identified and less frequent ones. These two variants are present in up to 20% and 15% in Caucasians, respectively, but much less common in Africans and Asians (Table 9.1). According to *in vitro* experiments with expressed mutant enzymes, the CYP2C9.2 and CYP2C9.3 proteins have reduced intrinsic clearance, although the degree of activity reduction appears to depend on the particular substrate. The **2* allele causes reductions in *in vitro* activity of ~20–40%, whereas the **3* allele is more severely affected and its activity reduction can be 70–90% for some substrates. In vivo, this results in clearance reductions of more than 70% in **3* homozygotes and in about half of the clearance for heterozygotes. Other alleles with decreased function but rare occurrence are **5, *6, *8, *11*, as well as several promoter variants with unclear significance.

Numerous studies demonstrated the clinical significance of the *CYP2C9*2* and **3* polymorphisms for most drug substrates mentioned above. Owing to their common occurrence in Caucasians, there are about 1–2% of homozygous (**2/*2* and **3/*3*) and hemizygous (**2/*3*) carriers that are at risk to experience more dramatic effects, but even for the heterozygous carriers, higher incidence of adverse drug reactions was

reported. This includes hypoglycemia as a result of treatment with hypoglycemic drugs [133], gastrointestinal bleeding from NSAIDs [134], and serious bleedings from warfarin treatment, where anticoagulant response also depends on variants of vitamin K epoxide reductase. Further examples of the clinical relevance of CYP2C9 polymorphisms can be found in recent reviews [131,135].

9.3.2.3.4 CYP2C19. Compared to CYP2C8 and CYP2C9, the CYP2C19 isozyme has a much lower expression in liver. However, it was the first CYP2C enzyme to be discovered by its marked genetic polymorphism resulting in the *S*-mephenytoin PM and EM phenotypes [120]. The CYP2C19 enzyme was later shown to be the major enzyme for the inactivating metabolism of proton pump inhibitors (PPI), including omeprazole and pantoprazole, and for the metabolic activation of the anticoagulant clopidogrel [136]. CYP2C19 has also a prominent role in the metabolism of several antidepressants of the first and second generation [137].

The PM phenotype results from two null alleles, that is, alleles that are unable to produce any functional protein, whereas EMs carry at least one functional allele. About 3–5% of white and black populations but up to 20% of Asians are CYP2C19 PMs, [6]. The two most common null alleles are *CYP2C19*2*, which occurs almost exclusively in Caucasians, and *CYP2C19*3,* which occurs primarily in Asians (Table 9.1). A further variant with potential clinical significance is a promoter variant *CYP2C19*17* that appears to be related to increased substrate turnover [138].

The effect of the *CYP2C19* polymorphism on *Helicobacter pylori* eradication therapy is a particularly intriguing example of a clinical application of pharmacogenetics. The common eradication strategy involves application of two antibiotics, for example, amoxicillin and clarithromycin, together with the PPI, which contributes to accelerated ulcer healing and increases effectiveness of the antibiotics. The PPI-induced increase in intragastric pH depends on the *CYP2C19* polymorphism. In several studies in Asia and Europe, it was shown that PM subjects benefit from their lower metabolism rate because their drug levels stay higher for a longer time [139].

Pharmacokinetic effects associated with *CYP2C19* genotype have also been reported for several antidepressants, including clomipramine [140], citalopram [141], and amitriptyline [142]. These were sometimes related to adverse side effects, but an influence on pharmacodynamic outcome remains so far controversial.

Several recent studies investigated *CYPC19* as a genetic determinant of the efficacy of the platelet-aggregation-inhibiting thienopyridine, clopidogrel (Plavix). This is important in the management of patients with coronary artery disease because a significant proportion of patients may be resistant and may experience insufficient platelet inhibition. The activation of clopidogrel by P450-dependent oxidations has been investigated, and several P450 enzymes, including the CYPs 2B6, 2C9, 2C19, and 3A4, have been found to contribute to the formation of the active 2-oxo metabolite [143]. Numerous clinical studies have confirmed that CYP2C19 PMs have significantly lower anticoagulation effect of clopidogrel, which is associated with an increased risk of major adverse cardiovascular events [144].

In conclusion, the CYP2C subfamily contains three active proteins CYP2C8, CYP2C9, and CYP2C19. Despite the number of common and functionally important variants in each gene being low (one or two), the clinical significance of these polymorphisms has been firmly established for a number of frequently used drugs. Given their strong relatedness in DNA and protein sequence (>82%) and common

mechanisms of transcriptional regulation, it is surprising how unique each enzyme is in terms of substrate specificity and clinical significance.

9.3.2.4 Subfamily CYP2D—CYP2D6.

9.3.2.4.1 Molecular Genetics and Pharmacological Properties. The *CYP2D* locus on chromosome 22q13.1 consists of the functional *CYP2D6* gene and two pseudogenes *CYP2D7* and *CYP2D8P*. While *CYP2D7* is expressed as mRNA in liver, only *CYP2D6* leads to functional protein and constitutes on average about 5% of the total P450 pool. Expression levels vary dramatically from person to person. A recent quantitative comparison of hepatic CYP2D6 protein by Western blot and mass spectrometric analyses revealed comparable results from undetectable in PMs up to 70 pmol/mg of microsomal protein and more in UMs [145]. At the RNA level, CYP2D6 expression is characterized by the occurrence of numerous splice variants. However, with the exception of the effect of polymorphic splice variants (see below), the functional significance of alternative splicing of CYP2D6 pre-mRNA has not been clarified. In fetal liver, CYP2D6 is virtually undetectable, but expression surges within hours after birth [146]. CYP2D6 is also expressed in several extrahepatic tissues, most notably in the gastrointestinal tract and in different areas of the human brain [147–149].

CYP2D6 metabolizes a large number of drugs from virtually all therapeutic classes, including analgesics, antiarrhythmics, antidepressants, antipsychotics, β-blockers, and anticancer drugs. Several highly selective test drugs have been used to determine the CYP2D6 drug oxidation phenotype, including debrisoquine, dextromethorphan, metoprolol, sparteine, and tramadol [150]. Endogenous biotransformations include 5-methoxyindolethylamine *O*-demethylase [151] and regeneration of serotonin from 5-methoxytryptamine [152]. The structure–activity relationships for CYP2D6 substrates and inhibitors were used to develop pharmacophore models [153]. Recently, the crystal structure of the CYP2D6 protein has been resolved, yielding further insights into the active site and the chemical requirements for binding and catalysis [154].

9.3.2.4.2 Genetic Polymorphisms and Pharmacogenetics. Among the human drug-metabolizing CYP1, 2, and 3 families, CYP2D6 shows the greatest impact of genetic polymorphism on expression and function and comparably little influence by environmental and nongenetic factors (except for drug–drug interactions). Initial evidence for *CYP2D6* genetic polymorphism came from population and family pharmacokinetic studies in the 1970s, which showed that deficient debrisoquine 4-hydroxylation [155] and sparteine N-oxidation [156] occur in 5–10% of European Caucasians as a monogenic recessive trait but at much lower frequencies in other ethnicities [157]. The molecular studies that followed have been reviewed in detail previously [6,148,158]. Currently, 78 distinct alleles and a large number of allele variants have been described, and the most relevant ones have been functionally assessed and shown to lead to absent or nonfunctional protein, or to decreased or increased expression (Table 9.1). The most frequent null alleles in Caucasians are haplotype variants of *CYP2D6*4*, which all harbor a consensus splice site mutation (1846G>A) that leads to absence of a detectable protein in the liver [159]. Its frequency among Caucasians is about 20–25%, and in white populations, it is responsible for 70–90% of all PMs [160,161]. The low frequency of the PM phenotype in Oriental and African populations is due to the virtual absence of the **4* allele, whereas in African-American populations, it is present with intermediate frequencies. The *CYP2D6* gene deletion allele **5* is present at a

frequency of 3–5% in most populations. The null alleles **3* and **6* are present at frequencies slightly above 1% in Caucasians, whereas the other null alleles are very rare [160,161]. In 10–15% of white individuals, the IM phenotype was shown to be the result of inheritance of the partially defective allele **41* in combination with another partially or fully defective allele. The mechanism involves an intron 6 SNP that leads to erroneous splicing resulting in only a fraction of correctly spliced mRNA [162].

In addition to the SNPs, a large number of structural variations exist at the *CYP2D* locus. Unequal crossing-over between the highly homologous genes involving a certain repetitive sequence also present in the *c-myc* gene leads to variants with deleted, duplicated, or recombined genes. *CYP2D6* gene duplications were identified in combination with various alleles including **1,*2, *4, *6, *10, *17, *29, *35,*41, *43*, and **45*, but most commonly, it occurs with the functional amino acid variant **2*. The overall frequency of the gene duplications in white Europeans is between 1% and 5%, whereas in some Arabian, Eastern African, and Pacific populations, it varies between 10% and over 50%, giving rise to speculations about possible selection processes, which might be related to the striking preference of CYP2D6 for plant alkaloids [158].

While PM alleles are very rare in Africans and Asians, other partially defective alleles termed **17* and **10*, respectively, are prevalent. The **17* allele is present at frequencies of up to 30% in Africans [163], and the **10* variant occurs at a frequency of up to 50% in Asians [164]. The partial activity conferred by these alleles leads to a shift in the metabolic drug oxidation capacity toward lower values, which has clinical relevance [165].

The presence of the pseudogenes, structural variants, and numerous SNPs at the *CYP2D* locus requires particular cautiousness in the design of genotyping assays. Coamplification of pseudogenes, unexpected recombination events, and failure to account for important variants, for example, due to ethnic variation, can lead to erroneous interpretation of genotype. Numerous genotyping assays and strategies were developed, which, because of the complexity of variants, usually identify only one functionally dominant key mutation per allele. This possibly reduces the predictive power of genotyping for certain haplotypes. The most comprehensive commercially available platform for CYP2D6 genotyping is the AmpliChip CYP450 test from Roche. This microarray has probes to identify 33 CYP2D6 alleles, including most confirmed variants responsible for absent or impaired enzyme activity and 7 gene duplications, as well as 2 *CYP2C19* variant alleles [166–168].

The *CYP2D6* polymorphism is one of the most intensely studied polymorphisms in man. It has been studied not only in relation to drug response for many CYP2D6 substrates but also as a risk factor for numerous diseases. Most of the epidemiological studies revealed conflicting results, and the reader is referred to specialized articles on the association of polymorphic *CYP2D6* alleles with, for example, Parkinson's disease [169], schizophrenia [170], Alzheimer's disease [171], and several forms of cancer [172–174].

In contrast to the largely inconsistent epidemiological studies, numerous clinical studies have shown profound effects of CYP2D6 polymorphism on pharmacokinetics, therapeutic effect, and associated toxicity of drugs that are either inactivated or activated by this enzyme. The limited scope of this chapter allows to mention only some of the newer studies, for example, on the impact of *CYP2D6* genotype on drug response following treatment with metoprolol [175,176], propafenone and other antiarrhythmics [177,178], and amitriptyline and other antidepressants [142,179–181]. An

important prodrug converted by CYP2D6 to the pharmacologically active substance is codeine, which is O-demethylated to morphine. Increased effectiveness of codeine with sometimes life-threatening opioid intoxication was observed in patients with multiple *CYP2D6* gene copies consistent with higher rates of conversion to morphine in patients with UM phenotype [182–184]. Recently, this scenario has also been the subject of a computerized quantitative modeling study [185].

CYP2D6 pharmacogenetics appears to have a major impact in the adjuvant treatment of breast cancer with tamoxifen, an antiestrogen extensively metabolized in the liver to several primary and secondary metabolites [186]. Formation of at least two metabolites, 4-hydroxytamoxifen and the secondary metabolite endoxifen, thought to be mainly responsible for the antiestrogenic effect because of their high affinity to the estrogen receptor depends mainly on the catalytic action of CYP2D6 [187]. Recent retrospective and prospective studies demonstrated that the CYP2D6 genotype significantly influences the plasma concentrations of these active tamoxifen metabolites, thus influencing treatment outcome [188]. According to these studies, patients with PM or IM phenotype produce lower levels of active metabolites and profit less from the treatment [77]. Nevertheless, it must be realized that the studies so far were limited by size and by the fact that confounding factors have not been taken into account systematically [189–191].

In conclusion, CYP2D6 is one of the cornerstones of pharmacogenetics and its translation into personalized medicine. The clinical significance of the *CYP2D6* polymorphism, which includes variants with completely deficient, decreased, and increased activity, has been firmly established for numerous frequently used drugs.

9.3.2.5 Subfamily CYP2E—CYP2E1. Among the human drug-metabolizing CYPs, CYP2E1 displays a substrate preference for low molecular weight molecules, including ethanol, acetone, pyrazole, acetaminophen, and many other toxicants and carcinogens, and thus is of primary toxicological importance, for example, for the metabolic activation of acetaminophen and many other chemicals [192]. The enzyme is inducible by many of its substrates, and in contrast to the transcriptional induction mechanisms, which play a role for most other drug-metabolizing P450s, CYP2E1 is induced post-translationally by substrate-induced stabilization. A number of studies also established the role of CYP2E1 in oxidative stress and alcoholic liver disease [193,194].

CYP2E1 is the only functional gene of the *CYP2E* subfamily, which is located at chromosome 10q26.3. Relatively few polymorphisms were described compared to other *CYP2* genes [7], but conclusive functional assessment of their phenotypic effects is lacking for most variants, and only moderate associations were described in studies with human liver [195–197]. Association with expression changes was reported for a polymorphic Rsa I site in the 5′-regulatory region of *CYP2E1*5* in one study [196] but not in another [195]. A recent study analyzed 11 polymorphisms and their haplotypes in over 2600 individuals from population samples representing the major geographical regions of the world [198]. As observed in studies on the CYP3A locus (see below), haplotype diversity was much higher in Africa (6–10 common haplotypes) than in other parts of the world (about 1–6 common haplotypes). This study also eluded on the difficulties in relating the haplotypes and alleles with different information content.

Because CYP2E1 is of particular relevance for metabolic activation of procarcinogens and chemical carcinogenesis, numerous pharmacogenetic studies were carried out

toward identifying variants associated with various cancers. As these studies are complex and often contradictory results were obtained, the reader is referred to some recent specialized reviews and meta-analyses on CYP2E1 and the risk of chemically induced cancers [199], its association with lung cancer risk [200], and alcohol-related cancers [201].

9.3.3 Family *CYP3*

9.3.3.1 Subfamily CYP3A—CYP3A4, CYP3A5, CYP3A7, and CYP3A43.

9.3.3.1.1 Molecular Genetics and Pharmacological Properties. The *CYP3A* cluster on human chromosome 7q22.1 has a size of 231 kb and comprises the four genes *3A4, 3A5, 3A7*, and *3A43*. The mouse cluster consists of twice as many genes, but there are no orthologous pairs between mouse and human, suggesting that a single *CYP3A* gene present in the common ancestor existed, which independently expanded during the last 75 million years [1]. CYP3A enzymes, in particular, CYP3A4, are responsible for the metabolism of ~30–40% of the clinically used drugs from all therapeutic categories. In part, this is explained by the structure of the enzymes. The active site of CYP3A4 is large and flexible enough to bind and metabolize many preferentially lipophilic compounds with comparatively large structures [202]. Typical substrates are, for example, the immunosuppressants cyclosporin A and tacrolimus; macrolide antibiotics, such as erythromycin; anticancer drugs, including taxol, cyclophosphamide, ifosfamide, and tamoxifen; benzodiazepines; the HMG-CoA reductase inhibitors simvastatin and atorvastatin; antidepressants; and opioids [203,204]. CYP3A4 is also an efficient steroid hydroxylase with an important role in the catabolism of several endogenous steroids including testosterone, progesterone, androstenedione, cortisol, and bile acids. The high sequence similarity between the CYP3A isozymes leads to highly similar substrate selectivity, so that no really selective probe substrates could be identified to date. Although several probe drugs that measure general CYP3A activity are available, including midazolam, erythromycin, alprazolam, and dextromethorphan [204], phenotyping for CYP3A is not a trivial task, as results obtained with different probe drugs are not generally well correlated to each other, which is a CYP3A4-specific feature that may be related to allosteric regulation of enzyme activity [205].

In the majority of individuals, CYP3A4 is the most abundantly expressed P450 in liver and intestinal enterocytes, as well as in colon and breast [63,84,206,207]. Expression of the three minor isoforms, CYP3A5, CYP3A7, and CYP3A43, is generally much lower than that of CYP3A4, although their exact contribution to the total CYP3A pool remained controversial [207–211]. CYP3A7 is more abundantly expressed in fetal liver than in adult liver, but the mechanism for this has not been studied in detail [146,212], whereas expression of CYP3A43 is negligible in liver [207]. Different signaling pathways contribute to the complex regulation of the *CYP3A* genes at the transcriptional level [203]. The xenosensor nuclear receptors PXR and CAR, on binding to structurally diverse drug ligands, including barbiturates, glucocorticoids, and rifampicin, are translocated to the nucleus, where they heterodimerize with RXR and enhance transcription from several PXR and CAR response elements located in the proximal promoter and in the xenobiotic-responsive enhancer module (XREM) region located −8 kb upstream [213,214]. By contrast, downregulation of CYP3A4 occurs in the course of inflammation [215]. Most CYP3A4 substrates show about 20–50% higher

in vivo clearance in females than males, and this difference has been explained by a higher hepatic expression at the mRNA, protein, and activity levels [216].

9.3.3.1.2 Genetic Polymorphisms and Pharmacogenetics.

CYP3A4. On the basis of a comparison of between- and within-person variances in CYP3A4 catalytic function, Ozdemir *et al*. [217] estimated a high inheritability of CYP3A4 catalytic function that warrants investigation into genetic polymorphism. Nevertheless, it should be noted that expression distribution was found to be unimodal, thus not indicating monogenic control. The CYPallele website currently lists 22 nonsynonymous variants, 3 SNPs resulting in frameshift, and a number of noncoding variants. Although several of the amino acid variants were shown to have an impact on catalytic function, their frequency in the general population is low so that homo- or hemizygotes are extremely rare, and therefore, only modest impact on *in vivo* pharmacokinetics can be expected. A more common and frequently studied polymorphism is the proximal promoter variant *CYP3A4*1B* [−392A>G], which occurs in white populations at ∼2–9% but at higher frequencies in subjects of African origin (Table 9.1). *CYP3A4*1B* was initially found to be associated with a higher tumor grade and stage in prostate cancer and showed higher nifedipine oxidase activity in human livers [218]. Association of *CYP3A4*1B* with markers of advanced disease was confirmed by some but not all further studies [219,220]. Despite additional *in vitro* studies with different substrates, the functional effect of this variant remained controversial [221–223]. Several systematic resequencing and haplotype tagging studies at the CYP3A locus carried out in ethnically diverse population samples essentially failed to discover any further variants of significant predictive power for CYP3A4 expression and/or function [208,209,220,224–226].

There are several possibilities to explain the apparent discrepancy between the expected high inheritability of CYP3A4 phenotype and the lack of predictive polymorphism in the *CYP3A4* gene. The importance of haplotype structures in the *CYP3A* locus is one possibility, but haplotypes have so far only been considered in few studies [225,227]. In one study, 37 tag SNPs distributed over the entire *CYP3A* locus in 5 ethnic groups were analyzed, and it was concluded that in European Caucasians, the *CYP3A* locus consists of the largest LD (linkage disequilibrium) blocks compared to other ethnic groups and that only 4 haplotypes account for over 80% of common European Caucasian haplotypes [228]. Haplotype diversity may therefore be more important in other ethnic groups, particularly of African origin. It is also possible that some important predictive variants have not been identified yet, as suggested by the recent discovery of an intronic SNP that appears to affect hepatic expression and response to statins [229]. Furthermore, the influence of genetic polymorphism may be masked or influenced by nongenetic effects. Evidence for the latter possibility was provided by an analysis that took the sex-dependent expression of CYP3A4 into account [227]. Finally, the genetic variants that determine CYP3A4 phenotype need not necessarily be located within the *CYP3A* locus. The various regulatory and signaling pathways that influence CYP3A4 expression may harbor variants that modulate constitutive or inducible transcription; for example, in nuclear receptors or their coactivators [230], drug transporters have been suggested to modulate CYP3A4-inducible expression by controlling the intracellular concentration of nuclear receptor ligands [231] and variants of the monooxygenase redox genes NADPH-CYP oxidoreductase and cytochrome *b5* may affect enzymatic activity [232]. Pathway-oriented gene approaches with a broad

coverage of genes in phenotypically well-defined tissues or volunteers may help to advance the pharmacogenomics of CYP3A4.

CYP3A5. In contrast to CYP3A4, expression of CYP3A5 in liver is polymorphic, and only a fraction of about 5–10% of Caucasians but 60% or more of Africans or African-Americans express this minor form. These ethnic differences are largely explained by two alleles that result in aberrant splicing and deficient expression. The most common allele is *CYP3A5*3*, which harbors an intron 3 mutation that leads to aberrant splicing and a truncated protein and occurs in all ethnic groups studied [211]. *CYP3A5*6* with an exon 7 mutation that also leads to an aberrantly spliced mRNA lacking exon 7 was only detected in populations of African origin. The high frequency of the *3A5*3* allele in Caucasians, compared to the lower frequency in Asian and African populations, explains the corresponding opposite frequency pattern of the functional *CYP3A5*1* reference allele that determines expression.

Pharmacogenetic studies addressing the CYP3A5 polymorphism have generally resulted in no or only moderate effects. This lack of penetrance is mainly due to the overlap in substrate selectivity among the CYP3A enzymes and to the minor expression of CYP3A5 compared to CYP3A4. Nevertheless, association with the *CYP3A5* genotype was reported, for example, for the immunosuppressant tacrolimus [233,234], the antihypertensive verapamil [235], and the HIV protease inhibitor saquinavir [236]. Further references on this subject and on the numerous studies on evaluation of CYP3A polymorphisms and cancer risk can be found in specialized reviews [211,237].

CYP3A7. The fetal predominant form CYP3A7 accounts for up to 50% of total P450 content in fetal livers [146,212]. Although expression shifts after birth from CYP3A7 to CYP3A4, it remains polymorphically expressed in some adult livers and in intestine [238]. Most of the CYP3A7 mRNA high expressor phenotypes could be explained by the *CYP3A7*1C* allele, which is more effectively transcribed due to several SNPs in the promoter region that lead to increased binding of and transactivation by PXR/RXR and CAR/RXR heterodimers to a polymorphic ER6 motif also found in the CYP3A4 promoter [238]. Further studies with a specific antibody estimated the relative content of CYP3A7 to the total CYP3A pool to be between 9% and 36% for the ~10% of high expressors and about 10-fold lower in the low expressors [239]. Additional alleles of the *CYP3A7* gene can be found on the CYPallele website [7]. The clinical significance of CYP3A7 polymorphism has not been well studied.

9.4 CONCLUSIONS AND FUTURE PERSPECTIVES

Among the 200 most frequently sold drugs in the United States, about 80% are cleared primarily by CYPs of the CYP1, CYP2, and CYP3 families [4]. The CYP1A2, CYP2C8, and CYP3A4 isoforms, which lack major functional polymorphisms, are responsible for the metabolism of about half of these drugs, whereas metabolism of the other half is by the polymorphic enzymes CYP2B6, CYP2C9, CYP2C19, and CYP2D6, which have an established role in pharmacogenetics [240]. An analysis of adverse drug reaction studies published between 1995 and 2000 identified 27 drugs frequently cited in connection with adverse drug reaction. While 59% of these drugs are metabolized

by at least one polymorphic enzyme, compared to only 7% of randomly selected top-selling drugs, emphasizing the clinical significance of pharmacokinetic polymorphisms [241]. For the major polymorphic human P450s, the most important functional variants have been identified and functionally characterized, although in some ethnically isolated populations, undiscovered important alleles may still exist. Impressive examples of genotype–phenotype or genotype–clinical outcome relationships have been elucidated that suggest advantageous clinical application. The less well predictable genes, especially CYP1A2 and CYP3A4, still pose a big challenge. Future studies need to consider pathways involved in their regulation and concepts for the inclusion of gene–gene and gene–environment interactions, as well as epigenetic aspects. Drug toxicity and treatment outcome depend on many additional genetic and nongenetic factors, and multigene and multifactorial approaches have to be considered more seriously in the future for successful application of the CYP polymorphisms in personalized drug therapy. Integration of such diverse data into comprehensive models of metabolic and gene regulatory networks poses future challenges for pharmacogenetics.

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