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Influence of cytochrome P450 polymorphisms on drug therapies: Pharmacogenetic, pharmacoepigenetic and clinical aspects

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Abstract

The polymorphic nature of the cytochrome P450 (CYP) genes affects individual drug response and adverse reactions to a great extent. This variation includes copy number variants (CNV), missense mutations, insertions and deletions, and mutations affecting gene expression and activity of mainly CYP2A6, CYP2B6, CYP2C9, CYP2C19 and CYP2D6, which have been extensively studied and well characterized. CYP1A2 and CYP3A4 expression varies significantly, and the cause has been suggested to be mainly of genetic origin but the exact molecular basis remains unknown. We present a review of the major polymorphic *CYP* alleles and conclude that this variability is of greatest importance for treatment with several antidepressants, antipsychotics, antiulcer drugs, anti-HIV drugs, anticoagulants, antidiabetics and the anticancer drug tamoxifen. We also present tables illustrating the relative importance of specific common *CYP* alleles for the extent of enzyme functionality. The field of pharmacoepigenetics has just opened, and we present recent examples wherein gene methylation influences the expression of CYP. In addition microRNA (miRNA) regulation of P450 has been described. Furthermore, this review updates the field with respect to regulatory initiatives and experience of predictive pharmacogenetic investigations in the clinics. It is concluded that the pharmacogenetic knowledge regarding CYP polymorphism now developed to a stage where it can be implemented in drug development and in clinical routine for specific drug treatments, thereby improving the drug response and reducing costs for drug treatment.

Keywords: Personalized medicine; Warfarin; Antidepressants; CYP2D6; Copy number variation; Tamoxifen

Contents

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1. Introduction

Pharmacogenetics is a field whereby the genetics of the individual patient is taken into consideration during drug development and for individualized therapies, overall improving the number of responders and decreasing the number of patients suffering from adverse drug reactions. Although factors like poor compliance, environmental factors and drug–drug interactions might affect the therapeutic outcome tremendously, and indeed more than the genetic factors, there are several examples wherein an altered gene constitution will influence the therapeutic outcome to such a large extent that it would not be ethically appropriate not to take these aspects into consideration as a physician. Also, during drug development, it is important to consider these aspects which could explain, or even prevent discarding of drug candidates if appropriate genetic reasons are identified, lack of response or occurrence of ADRs in drug therapy.

In general one can envision important pharmacogenetic variation at the level of

- drug transporters,
- drug metabolizing enzymes,
- drug targets,
- other biomarker genes

As being important for the interindividual differences in drug response. So far, it is apparent that variability in genes encoding drug metabolizing enzymes often affects outcome in drug treatment to a very high extent and that the polymorphism of the cytochrome P450 (CYP) enzymes plays a major role in this respect. Because of such variability, the populations could be classified into 3 major phenotypes:

- the ultrarapid metabolizers (UM), with more than 2 active genes encoding a certain P450;
- the extensive metabolizers (EM), carrying 2 functional genes; and
- the poor metabolizers (PM), lacking functional enzyme due to defective or deleted genes.

In addition, a more subtle phenotype occur that is commonly called

- the intermediate metabolizers (IM), usually carrying 1 functional and 1 defective allele but may also carry 2 partially defective alleles.

By contrast, polymorphism in genes encoding drug transporters and drug receptors do, in some cases, influence therapeutic outcome, but the number of important examples where this variation is of clinical importance are fewer.

Thus, with respect to the penetrance of polymorphic genes on drug disposition and action, it is evident that the genes encoding drug metabolizing enzymes exhibit a prominent role because of the great influence on drug elimination, thereby influencing the effect of drugs in the treatment of many different diseases. In general, it can be estimated that 20–25% of all drug therapies are influenced by such polymorphism to an extent that therapy outcome is affected (Ingelman-Sundberg, 2004) and the CYP play a critical role, as these enzymes are responsible for about 80% of all phase I drug metabolism (Eichelbaum et al., 2006). This makes the field of CYP pharmacogenetics of great importance both for drug development and for drug treatment in clinical practice.

Recently, some reviews have covered the topic regarding the use of pharmacogenetics in drug treatment (Ingelman-Sundberg, 2004; Eichelbaum et al., 2006; Gardiner & Begg, 2006). In the present review, we update the field on CYP pharmacogenetics and focus on novel pharmacogenetic aspects concerning gene copy number variation, epigenetics, as well as the

implementation of knowledge into drug development and clinical use.

2. Molecular aspects of cytochrome P450 genetic polymorphism

2.1. Overview

The human CYP genes are highly polymorphic. The different alleles are summarized at the Human CYP allele nomenclature committee home page (www.cypalleles.ki.se) present on a server at Karolinska Institutet. The Web site currently encompasses alleles described for the *CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2A13, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP2F1, CYP2J2, CYP2R1, CYP2S1, CYP3A4, CYP3A5, CYP3A7, CYP3A43, CYP4A11, CYP4A22, CYP4B1, CYP5A1, CYP8A1, CYP19A1, CYP21A2* and *CYP26A1* genes. All new alleles submitted to the CYP allele Web site are peer reviewed before designation of allele names and publication on the Web site based on the fulfillment of the general inclusion criteria given at www.cypalleles.ki.se/criteria. At present, more than 350 functionally different *CYP* alleles, i.e., gene variants that affect the function and/or activity of the gene products, are presented at the CYP allele Web site, wherein the highest number of variant alleles are described for *CYP2D6* (63 alleles), *CYP2B6* (28), *CYP1B1* (26) and *CYP2A6* (22). Notably, no common variant allele affecting function has been described for *CYP2E1* and only a few for *CYP2R1* and *CYP2S1*, possibly because of high extent of conservation during evolution due to important endogenous functions. In order to visualize the actual importance of the various *CYP* alleles as judged by the users of the Web site, we show in Fig. 1 the relative number of visits for the various *CYP* alleles. It appears that *CYP2D6* and *CYP2C9* are the 2 genes on which the users, both from academia and industry, focus most of their attention.

The functional *CYP* polymorphisms consist of gene deletions, gene duplications, and deleterious mutations creating inactive gene products, e.g., small insertions and deletions causing frame shift mutations, etc. Furthermore, amino acid changes might be introduced which, in some cases, can change the substrate specificity. Not only mutations in the open reading frame are important with respect to altered function of the *CYP* genes, but also many examples where, e.g., mutations in introns creating altered splice sites are apparent. An important aspect of the CYP polymorphism is the copy number variation where multiple functional gene copies of one allele can result in increased drug metabolism and absence of drug response at ordinary dosage. This aspect has recently been emphasized because of the development of efficient methods determining the whole human genome copy number variants (CNV; Redon et al., 2006; Stranger et al., 2007).

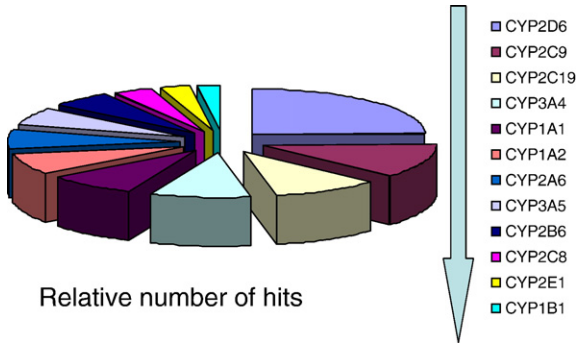


Fig. 1. Importance of the different polymorphic CYP genes as assessed by the number of hits at the human CYP allele nomenclature home page (www.cypalleles.ki.se).

2.2. Copy number variation

CNV are DNA segments at 1 kb or larger with a variable number of copies in comparison with a reference genome. CNV can have dramatic phenotypic consequences as a result of altering gene dosage, disrupting coding sequences, or perturbing long-range gene regulation (Stranger et al., 2007). Initially CNV were thought to be very rare events. An increased number of functionally active genes was first described for CYP2D6 (Bertilsson et al., 1993; Johansson et al., 1993) where alleles carrying from 0 to 13 gene copies were described to be stably inherited, and in the next decade, not many more examples were given. By the invention of new techniques that rapidly allow the analysis of full genomic occurrence of CNVs using single-nucleotide polymorphism (SNP) genotyping arrays and clone-based comparative genomic hybridization, it was recently found that this is a very common phenomenon in the human genome. A total number of 1447 CNV had been identified in the human genome, covering 360 Mb (12% of the genome), and more than half of them (58%) overlap known RefSeq genes, with duplications being significantly more frequent than deletions in these CNV overlapping genes (Redon et al., 2006; Stranger et al., 2007). When studying the functional categories enriched with CNV, cell adhesion, sensory perception of smell and chemical stimulus are overrepresented. On the other hand, cell signaling, cell proliferation, and kinase and phosphorylation-related categories are underrepresented, suggesting that these classes of genes are more likely to be dosage sensitive (Redon et al., 2006). Focus on enzymes involved in xenobiotic metabolism reveals that there are several well-known examples of CNV, including *CYP2A6, CYP2D6, GSTM1, GSTT1, SULT1A1, SULT1A3, UGT2B17,* and also the nearby *UGT2B7, UGT2B10* and *UGT2B11 genes*. All these genes are deleted at a relatively high frequency in at least one ethnic group. In addition, *CYP2A6, CYP2D6, SULT1A1* and *SULT1A3* can also present duplications and even multiduplications (Johansson et al., 1993; Hildebrandt et al., 2004; Fukami et al., 2007; Hebbring et al., 2007; Fig. 2). The *CYP2A6* and *CYP2D6* CNV affect drug metabolism to a large extent, but do not crucially affect disease susceptibility, although due to the role of CYP2A6 in nicotine metabolism and the activation of carcinogens, the CYP2A6 CNV might influence cancer risk and nicotine intake (Kamataki et al., 2005; Malaiyandi et al., 2005). Thus, because the drug metabolizing enzyme CNVs do not result in disease, a high frequency is permitted. On the other hand, CNV in CYP genes of endogenous importance cause disease and are consequently rare (e.g., *CYP21A2* and

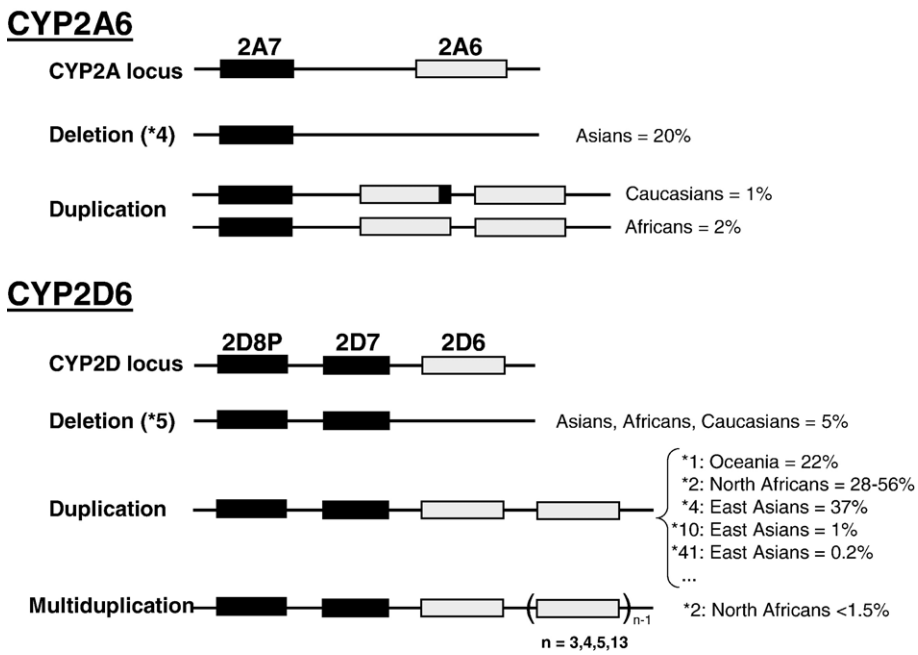


Fig. 2. CNV affecting CYP genes. The *CYP2A* and *CYP2D* gene clusters on chromosomes 19 and 22, respectively, are shown. Pseudogenes are shown in black, while *CYP2A6* and *CYP2D6* genes are shown in grey. Frequent gene deletions, duplications and multiplications are depicted and the population in which the highest frequency has been described is shown on the right (expressed in percentage).

congenital adrenal hyperplasia; cf. Wilson et al., 2007). There is also a large interethnic variation in the CNV of metabolizing genes, for example, *CYP2A6* deletion (*CYP2A6*4*) has a higher frequency in Asians (7–24%) than in Caucasians and Africans (about 1%), *CYP2D6* deletion (*CYP2D6*5*) has similar frequency (about 4%) in the different populations, but *CYP2D6* duplication/multiduplication is more frequent in Africans than in Asians and north Caucasians (Fig. 2). The CNV in drug-metabolizing CYP have been suggested to be subjected to selection pressure to adapt to a changing dietary environment (Ingelman-Sundberg, 2005).

2.3. Pharmacoepigenetics and microRNAs

Interindividual variability in CYP expression could be envisioned to be controlled by microRNAs (miRNAs), and thus, genetic changes at the mRNA target binding sites or at the miRNA precursor could contribute to a variable CYP expression. In addition to genetic factors, epigenetic control of CYP expression could also play a role by differences in gene methylation. These 2 mechanisms could be crucial for the regulation of CYP expression and could have special relevance for those *CYP* genes exhibiting interindividual variation but where no genetic variants affecting their promoter, splicing and coding regions have been identified. Thus, miRNAs and epigenetic mechanisms could underlie the variability in the expression of some CYPs in which important genetic variants have failed to be identified.

2.3.1. Control of cytochrome P450 gene methylation

Methylation of DNA is regarded as a means of regulating gene expression through 2 general mechanisms. First, DNA methylation of gene promoters may reject the binding of some transcription factors to their DNA binding sites (Tate & Bird, 1993; Rountree et al., 2001). Second, the transcriptional silencing capability of DNA methylation may occur via complex indirect mechanisms involving changes in chromatin conformation (Keshet et al., 1986; Nan et al., 1998; Stirzaker et al., 2004; Caiafa & Zampieri, 2005; Padjen et al., 2005). The interaction of methylated DNA with proteins that detect methylated DNA and other chromatin remodeling proteins render an altered chromatin configuration that prevents the expression of a gene (Rountree et al., 2001).

DNA methylation occurs predominantly at CpG sites in the mammalian genome (Jones & Takai, 2001) by the DNA methyltransferase (DNMT) enzymes (Bestor, 2000; Rountree et al., 2001). Of the 4 bases, only cytosine has a physiologically modified analogue, 5-methylcytosine, which is methylated at position C5 of the pyrimidine ring (Shiraishi et al., 2002). Three to four percent of all cytosines are methylated, and the resulting 5-methylcytosines make up 0.75–1% of all nucleotide bases in the DNA of normal human tissue (Esteller, 2003). High concentrations of these CpG dinucleotides, called CpG islands (Gardiner-Garden & Frommer, 1987), can be found most often at promoter regions and first exons but may also occur in regions more toward the 3′ end (Bird, 1980; Larsen et al., 1992; Jones & Takai, 2001; Rountree et al., 2001).

Expression of some members of the CYP family has been shown to be affected by changes in DNA methylation and appears to occur mainly in genes of importance for the metabolism of endogenous compounds. Aberrant alterations in the methylation status of CpG dinucleotides, target sites for DNMT, have been shown in the promoter and enhancer regions of CYP1B1 in prostate cancer (Tokizane et al., 2005). The

activation of CYP1B1 via the AhR/ARNT pathway requires the recognition of the dioxin response element (DRE; also called xenobiotic response element, XRE; GCGTG) which contains the DNMT target dinucleotide. Comparison of CYP1B1 expression in benign prostatic hyperplasia (BPH) and prostate cancer reveals increased levels in the latter. This change is linked with demethylation of the CpGs in the DRE in prostate cancer. Likewise, the transcription factor Sp1 binding site GGGCGG was demethylated in the prostate cancer tissues but remain methylated in BPH. This study suggests that hypomethylation of sites in the promoter and enhancer regions of CYP1B1 allow the binding of transcription factors and promote the expression of the *CYP1B1* gene in prostate cancer (Tokizane et al., 2005).

Whereas hypomethylation of the *CYP1B1* promoter in prostate cancer allows for its expression, *CYP1A1* has been shown to be silenced due to hypermethylation (Okino et al., 2006). The *CYP1A1* regulatory region, which contains a CpG island, is methylated in the prostate cancer cell line LNCaP but not in the noncancerous cell line RWPE-1. This aberrant methylation has also been shown to affect the local chromatin structure by the modification of histones. Trimethylated lysine 4 in histone H3 (me3H3K4), which is exclusively associated with active genes, is depleted in the DNA regions containing DRE in LNCaP. This altered chromatin structure is likely associated with the inability of the AhR/ARNT complex to interact with these sites (Okino et al., 2006). The earlier work of Anttila et al. (2003) has also suggested the role of DNA methylation in CYP1A1 regulation. In this study, it was shown that lifestyle (i.e., smoking) can afford changes in *CYP1A1* DNA methylation in such a way that smokers have lower methylation levels than nonsmokers and that methylation increased after 1–7 days of quitting smoking. The aberrant DNA methylation that occurs in cancer cells also affects the expression of the novel P450 member CYP2W1 (Gomez et al., 2007). We have also shown that the differences in CYP2W1 expression level in human-derived cell lines is due to variation in the methylation status of the CpG island in the proximal promoter of the *CYP2W1* gene (Karlgren et al., 2006).

In Fig. 3, we have made an analysis of the possible methylation sites, as evident from the presence of CpG islands, in CYP genes. Besides *CYP1A1*, *CYP1B1* and *CYP2W1*, also *CYP1A2*, *CYP2C19*, *CYP2D6*, *CYP2E1*, *CYP2J2*, *CYP2R1* and *CYP2S1*, have potentially functional methylation sites. Future research would tell us to what extent these sites are subject for regulatory control.

The significance of epigenetics in drug therapy has been demonstrated in the epigenetic silencing of *CYP24*, the key enzyme responsible for vitamin D catabolism. Chung et al. (2007) have shown that endothelial cells derived from different microenvironments (tumor versus normal) have different CYP24 promoter methylation status. This causes differences in response to calcitriol, the most active form of vitamin D, in that endothelial cells that possessed promoter-methylated CYP24 were recalcitrant to calcitriol induction and were also more susceptible to the growth inhibitory effect of calcitriol. Response to the anticancer agent tamoxifen, a selective estrogen receptor modulator which has been shown to dramatically reduce the risk of breast cancer and of breast cancer recurrence, may also depend on the expression of the epigenetically

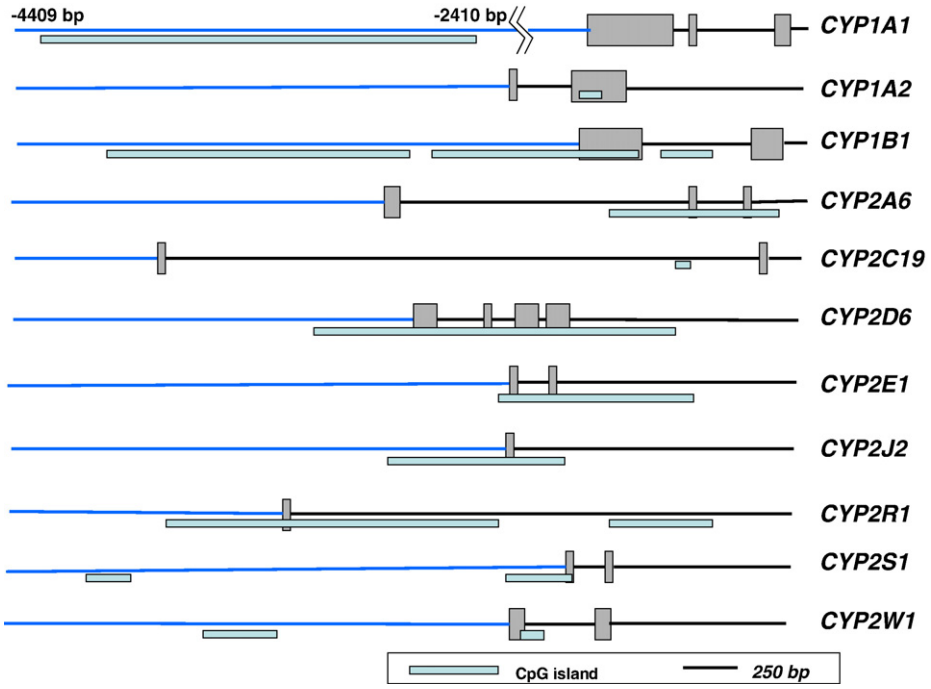


Fig. 3. CpG islands in promoter and 5′ regions of drug-metabolizing members of the CYP family. Prediction of CpG islands was performed using Methyl Primer Express, version 1.0 (Applied Biosystems). CpG islands are defined as stretches of DNA with at least 200 bp in length, at least 50% C+G content, and at least 0.6 Observed/Expected CpG dinucleotides (Gardiner-Garden & Frommer, 1987). Medium grey boxes, exons; light blue/grey long boxes, CpG islands. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

regulated CYP1B1 since it metabolizes tamoxifen and estradiol. Widschwendter et al. (2004) suggested that the extent of *CYP1B1* methylation could predict survival in tamoxifen-treated and nontreated patients, an observation that however needs to be confirmed.

The early work of Jones et al. (1992) investigated the possible involvement of DNA methylation of the *CYP2E1* gene in human development but found no conclusive evidence. In rat development, on the other hand, Umeno et al. (1988) have shown that specific demethylation at the 5′ end of the *CYP2E1* gene obtained from the liver coincided with transcriptional activation during early development. They have shown that the increase in CYP2E1 expression up to 7 days after birth is correlated with demethylation at the 5′ region, but further demethylation up to 10 weeks was not associated with gene activity. The work of Botto et al. (1994) aimed to analyze variations in CYP2E1 expression in various human adult tissues and to explain this variation by DNA methylation. Differential methylation in various tissues was observed, and hypomethylation of *CYP2E1* gene corresponded with a decreased expression of the protein. Since this relationship does not follow the conventionally accepted knowledge that DNA methylation induces silencing of genes, it may be possible that demethylated DNA is open for binding of repressive proteins. Vieira et al. (1996) reported an association between CYP2E1 transcripts and decreased methylation of CpG residues in intron 1 of the *CYP2E1* gene that occurred during the late neonatal period.

Epigenetic events are very flexible. In the study by Fraga et al. (2005), it was shown that the environment can have an effect on the epigenetic landscape of an individual, as well as his/her gene expression profile. In a mice experiment by Cooney et al. (2002), they also showed that diet can have an effect on DNA methylation. This can also have an influence on the response to therapeutic treatments and thus might possibly be considered in clinical practice.

2.3.2. MicroRNA regulation

miRNAs are a family of noncoding RNAs that base-pair to target mRNAs and typically decrease their expression (Massirer & Pasquinelli, 2006). miRNAs regulate genes involved in various cell processes like proliferation, morphogenesis, apoptosis and differentiation.

In animals, miRNAs are processed from primary transcripts known as pri-miRNAs or pri-miR. These are then processed into a ~60 bp hairpin precursor called pre-miRNA or pre-miR, which are further processed into the mature forms that are ~22 bp in length. This sequential cutting is done by 2 RNase III enzymes, Drosha and Dicer. miRNAs are found in plants and animals but not in fungi (Berezikov et al., 2006; Carthew, 2006). They control the expression of a gene by targeting the 3′-UTR of mRNAs and result ultimately in the degradation of the mRNA. This occurs through the formation of a ribonucleoprotein complex called RNA-induced silencing complex (RISC) that guides the recognition and translational repression or degradation of target mRNAs. In many cases, target mRNA levels diminish but do not disappear (Farh et al., 2005; Lim et al., 2005).

At present, about 0.5–1.5% of the total genes in sequenced animal species are known miRNA genes (Carthew, 2006). There are at least 474 known miRNA genes in humans, 78 in *Drosophila melanogaster* and 132 in *Caenorhabditis elegans* (see http://microrna.sanger.ac.uk/cgi-bin/sequences/browse.pl). However, these numbers are rapidly increasing with the continued application of both genomic and bioinformatic approaches.

miRNA regulation of CYPs has hitherto only been identified for CYP1B1, and Tsuchiya et al. (2006) showed the involvement of miRNA in the regulation of this gene expression. Previous studies have shown greater expression levels of CYP1B1 protein in various types of malignant cancers compared with normal tissues, whereby the CYP1B1 enzyme has been associated with cancer. The CYP1B1 transcript has an especially long 3′-UTR with regions of extreme interspecies conservation, which were suggestive of potential miRNA regulation (Table 1). Tsuchiya et al. discovered a region in the 3′-UTR with near-perfect complementarity to miR-27b, which they then validated through several complimentary routes of experimentation. This forerunning study of miRNA regulation of CYP1B1 strongly supports a potential and significant role for miRNAs in the regulation of drug metabolizing enzymes. Furthermore, this additional layer of post-transcriptional regulation could be responsible for a portion of the significant amount of unexplained interindividual variability in enzyme expression and activity.

The probability of potential sites for miRNA regulation of CYPs is higher if the gene contains a longer 3′-UTR region. In Table 1, we show the length of the 3′-UTR regions based on the genomic reference sequences among the most important *CYP* genes encoding drug and xenobiotic metabolizing enzymes, and it is apparent that CYP1A2, CYP2B6, CYP2S1 and CYP3A4,

Table 1
Size of the 3′-UTR regions (based on the definition in the reference sequences) and the number and location of CpG islands of possible regulatory function in the genes of the human CYPs in the 1–3 families

Gene	Size of 3′-UTR (bp)	Number of CpG islands	Location of the CpG islands	Reference sequence
CYP1A1	940	1	5′-flank	NC_000015.8
CYP1A2	1512	1	Intron 1	NC_000015.8
CYP1B1	3119	3	5′-flank, exon1, intron 1	NC_000002.10
CYP2A6	257	1	Intron 1-intron 3	NC_000019.8
CYP2A13	253	0		NC_000019.8
CYP2B6	1569	0		NC_000019.8
CYP2C8	356	0		NC_000010.9
CYP2C9	362	0		NC_000010.9
CYP2C18	323	nd		NC_000010.9
CYP2C19	318[a]	1	Intron 1	L39102.1
CYP2D6	75	1	5′-flank-intron 4	NC_000022.9
CYP2E1	152	1	Exon 1-intron 2	NC_000010.9
CYP2R1	113	2	5′-flank-intron 1	NC_000011.8
CYP2S1	1051	2	5′-flank	NC_000019.8
CYP2W1	818	2	5′-flank, intron 1	NC_000007.12
CYP3A4	1152	0		NC_000007.12
CYP3A5	111	0		NC_000007.12
CYP3A7	463	0		NC_000007.12

n.d. = none detected.
[a] The only available 3′ sequence information (clone of exon 9). The 3′-UTR may be longer.

besides CYP1B1, is more likely to be regulated by miRNAs. Further research is needed to validate this point.

2.4. Cytochrome P450 polymorphism

As mentioned, many of the genes encoding CYPs involved in drug metabolism are highly polymorphic. The most important polymorphic enzymes in this respect are CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and the CYP3As. In this chapter, we give an update on the issue of their polymorphism with emphasis on novel aspects of clinical importance.

2.4.1. CYP1A2

CYP1A2 is a surprisingly well-conserved gene. With respect to variant alleles, there are no common alleles described that cause any important alteration in gene expression or enzyme activity. Concerning mutations in the regulatory regions, alleles *CYP1A2*1F* and *CYP1A2*1K* have received interest. The *CYP1A2*1F* allele contains a 163C>T mutation in intron 1, and this has been shown in several independent studies to influence the inducibility of the gene and affect the magnitude of increase of in vivo caffeine metabolism after both smoking (Sachse et al., 1999; Ghotbi et al., 2007) and omeprazole treatment (Han et al., 2002). The molecular mechanism behind this has not been described and awaits further research. A variant of this allele has been found in African populations (Aklillu et al., 2003), and besides 163C>T, it contains −729C>T, which abolishes a binding site for an Ets nuclear factor resulting in highly decreased CYP1A2 expression and caffeine metabolism. This allele (*CYP1A2*1K*) is however relatively rare. In contrast to the few variant genes identified, the interindividual variation in CYP1A2 activity as assessed by, e.g., in vivo caffeine metabolism is extensive and no polymorphic site in the *CYP1A2* gene that can unequivocally be used to predict the metabolic phenotype in any individual has yet been identified despite quite ambitious sequencing efforts (Jiang et al., 2006). One might speculate that other possibly epigenetic phenomena or polymorphism in *trans*-acting genes could be explanations for the genetic part of such interindividual variability. Despite the absence of any functional gene variants, many epidemiological association studies have been published trying to link particular polymorphic sites to disease susceptibility like, e.g., various forms of cancer, myocardial infarction, tardive dyskinesia and schizophrenia, as well as risk of recurrent pregnancy loss. Such association studies has hitherto not provided with much consensus information.

2.4.2. CYP2A6

CYP2A6 is mainly expressed in the liver and in trace amounts in extrahepatic tissues. It metabolizes several important therapeutic drugs, toxins and procarcinogens, as well as nicotine and its metabolite cotinine (Xu et al., 2002). In this respect, a high CYP2A6 expression has been proposed to increase the susceptibility to nicotine addiction and the risk of tobacco-related cancers (Kamataki et al., 2005; Malaiyandi et al., 2005). CYP2A6 activity in vitro an in vivo can be estimated easily by measuring coumarin oxidation, and with this method, a very large interindividual variability in CYP2A6 activity (up to 300-fold) has been reported. Interestingly, the CYP2A6 interindividual variation shows important ethnic differences, with only 1% of Caucasians being PM, whereas this figure is up to 20% in Asians. *CYP2A6* expression can only be modestly induced, suggesting that environmental factors are not important for the observed CYP2A6 variability. In contrast, genetic variants have been shown to influence its expression and/or activity. Up to date, several polymorphisms with functional significance have been identified. Two relatively common alleles, *CYP2A6*2* and *CYP2A6*4*, as well as *CYP2A6*5* and *CYP2A6*20*, result in an abolished activity of the enzyme. In *CYP2A6*2*, a single amino acid change renders the enzyme inactive, whereas *CYP2A6*4* has a gene deletion that accounts for the majority of PM in the Asian population (see www.cypalleles.ki.se). Eight *CYP2A6* alleles (**6*, **7*, **10*, **11*, **12*, **17*, **18* and **19*) lead to enzymes with reduced activity, and 3 genetic polymorphisms in the promoter region of *CYP2A6* have reduced transcriptional activity (alleles **1D*, **1H*, **9*). Because the conversion of nicotine to cotinine is mediated by *CYP2A6*, the effect of these alleles has been substantially studied with respect to smoking behavior and nicotine dependence, suggesting that *CYP2A6* genetic variation could play a role in smoking and tobacco-related cancer risks (Kamataki et al., 2005; Malaiyandi et al., 2005), although there appears to be a need to reproduce these findings in larger cohorts. The *CYP2A6*1B* allele, having a gene conversion in the 3′-flanking region with *CYP2A7*, has been shown to be expressed at higher levels than *CYP2A6*1A* and to cause a more rapid nicotine metabolism (Nakajima et al., 2001; Mwenifumbo et al., 2007). The 3′ gene conversion is also present in several additional *CYP2A6* alleles (see www.cypalleles.ki.se).

2.4.3. CYP2B6

CYP2B6 was initially considered not to be of importance in drug metabolism, but new investigations indicate high relevance of this enzyme in the metabolism of, e.g., anticancer drugs, like cyclophosphamide and ifosfamide, and anti-HIV drugs, like efavirenz and nevirapine (see www.hiv-pharmacogenomics.org; Owen et al., 2006; Turpeinen et al., 2006 for recent reviews). Recently, the importance of CYP2B6 in the metabolism of environmental chemicals has been emphasized (Hodgson & Rose, 2007). The human *CYP2B6* gene is highly polymorphic. However, no CNVs have been identified, nor any mutation creating an important loss of function except for the rare *CYP2B6*28* allele carrying 1132C>T, that results in protein truncation at arginine 378 (Rotger et al., 2007). There appears to be a couple of variant alleles that are associated with lower expression/activity, and these are *CYP2B6*6*, *CYP2B6*16* and *CYP2B6*18* in particular (Tsuchiya et al., 2004; Wang et al., 2006b; Rotger et al., 2007). Of these, *CYP2B6*6* is rather common in several different populations (20–30% frequency), whereas both *CYP2B6*16* and *CYP2B6*18* are common in Black subjects where the allele frequency is relatively high (7–9%; Wang et al., 2006b; Rotger et al., 2007). The *CYP2B6*16* allele is expressed less efficiently in

heterologous systems, whereas the molecular basis behind the altered activity in individuals carrying *CYP2B6*18* has not been clarified. In clinical studies, it is apparent that subjects homozygous for combinations of the alleles *CYP2B6*6*, *CYP2B6*16* and *CYP2B6*18* exhibit lower capacity for metabolism of CYP2B6 substrates, like efavirenz, than expected from a linear gene–dose relationship (Wang et al., 2006b; Rotger et al., 2007). In addition, the *CYP2B6*6* allele appears to cause both high and low activity of CYP2B6 in different studies, and it is conceivable that the 516G>T and 785A>G mutations, causing the amino acid substitutions, Q172H and K262R are linked to other mutations, giving rise to specific haplotypes that are associated to high or low activity of CYP2B6. The *CYP2B6*4* allele has been implicated to cause higher V_{max} values both in vitro (Jinno et al., 2003) and in vivo (Kirchheiner et al., 2003). Overall, there is a marked interindividual variability in the CYP2B6 activity, but the current pharmacogenetic knowledge is not sufficient as to provide with efficient tools to predict of the specific capacity for metabolism of CYP2B6 substrates. Most alterations in function of the various alleles have only been consistently observed in in vitro systems, and haplotype characterization at a higher resolution in this locus is necessary before any clinically relevant predictions about an individual's CYP2B6 activity can be given.

2.4.4. CYP2C8

The *CYP2C8* gene is located at chromosome 10q24 in a multigene cluster containing the other *CYP2C* subfamily members *CYP2C9*, *CYP2C18* and *CYP2C19*. From these highly homologous genes, *CYP2C8*, *CYP2C9* and *CYP2C19* are clinically relevant and exhibit functionally important genetic polymorphisms that confer differences in the metabolism of CYP2C substrates. CYP2C8 is expressed mainly in the liver where it participates in the metabolism of important drugs for the treatment of diabetes (repaglinide, rosiglitazone, troglitazone, pioglitazone), cancer (paclitaxel, all-*trans* retinoic acid), malaria (amodiaquine and chloroquine) and arrhythmias (amiodarone, dapsone), among others. Concerning extrahepatic expression, CYP2C8 oxidizes the endogenous substrate arachidonic acid to vasoactive derivatives, and it has been proposed that CYP2C8 might be relevant for the development of cardiovascular diseases (Yasar et al., 2003; Lundblad et al., 2005). CYP2C8 is PXR- and CAR-inducible through a distal site located at −8 kb in the promoter (Ferguson et al., 2005). Constitutively, hepatic CYP2C8 is expressed at somewhat lower levels than CYP2C9 but at higher levels than CYP2C19 (Edwards et al., 1998). In contrast to CYP2C9 and CYP2C19, the molecular mechanisms involved in CYP2C8 interindividual variation are still unclear, probably because CYP2C8 was previously considered to play a minor role in drug metabolism, therefore decreasing the interest to study this gene. In 2001, the CYP2C8 substrate cerivastatin was withdrawn from the market due to severe or even fatal rhabdomyolysis, providing evidence for the relevance of CYP2C8 drug interactions. Many of the cerivastatin adverse effect reports showed coadministration of gemfibrozil, a CYP2C8 inhibitor, and nowadays, there are evidence that an altered CYP2C8 activity contributed, at some extent, to cerivastatin toxicity (Wang et al., 2002; Ishikawa et al., 2004; Ozaki et al., 2005). In addition, CYP2C8 plays a major role in the inactivation and elimination of paclitaxel, a widely used anticancer treatment that, as many other anticancer drugs, has a narrow therapeutic index and frequent severe toxicities (Taniguchi et al., 2005).

The variation in the metabolism of CYP2C8 substrates in vitro and in CYP2C8 expression is large (Bahadur et al., 2002); however, it is difficult to estimate the in vivo variation, since there is no standardized test to phenotype CYP2C8 activity. Paclitaxel pharmacokinetics, through the CYP2C8 mediated 6α-hydroxylation of the molecule, likely corresponds to CYP2C8 activity (Taniguchi et al., 2005); however, due to its toxicity, it is not a desirable probe drug for phenotyping. There is large interindividual difference in paclitaxel pharmacokinetics, with the individual clearance range of unbound paclitaxel varying from 83.7 to 1055 L/hr in 97 cancer patients (Henningsson et al., 2005). The pharmacokinetics of the antidiabetic repaglinide, which is a drug actively metabolized by CYP2C8, have been measured in vivo in healthy volunteers; however, it has been shown that the major determinants for repaglinide pharmacokinetics are polymorphisms affecting the hepatic uptake transporter OATP1B1, and thus, it can not be used in vivo as a CYP2C8 probe drug (Niemi et al., 2005).

To date, several coding region SNPs have been described in the *CYP2C8* gene (www.cypalleles.ki.se) with important interethnic variations: *CYP2C8*2* is present mainly in Africans, whereas *CYP2C8*3* and *CYP2C8*4* are mainly distributed in Caucasians (Soyama et al., 2002b; Weise et al., 2004; Cavaco et al., 2005). Other variants leading to amino acid changes are extremely rare. In addition, there are 2 SNPs described in the promoter region of *CYP2C8* (**1B* and **1C*; Bahadur et al., 2002). Except for the rare *CYP2C8*5* that encodes a truncated protein, the contribution of the described *CYP2C8* alleles to the observed interindividual variability remains unclear.

*CYP2C8*3* results in 2 amino acid substitutions (R139K and K399R), which have been reported to be in total linkage disequilibrium; however, 2 studies have reported individuals that carry only one of these SNPs (Taniguchi et al., 2005; Daly et al., 2007). In vitro studies using heterologous proteins found that CYP2C8.3 had a reduced activity using paclitaxel and arachidonic acid as substrates, but there were no differences for amiodarone metabolism. Depending on the expression system used, the activity of CYP2C8.3 for paclitaxel metabolism was 15% and 75% of the wild-type activity for bacteria and mammalian cells, respectively (Dai et al., 2001; Soyama et al., 2001, 2002a, 2002b). CYP2C8.2 has only been expressed in bacteria with a reduction to 55% of the turnover of the wild-type enzyme using paclitaxel (Dai et al., 2001) and for amodiaquine, a 3-fold higher Km and 6-fold lower intrinsic clearance has been shown (Parikh et al., 2007), whereas CYP2C8.4 has not been expressed in heterologous systems. With respect to human liver microsomes, a small decrease in paclitaxel 6α-hydroxylation was found for *CYP2C8*3*-positive samples, but no differences were found for the *CYP2C8*4* livers (Bahadur et al., 2002). In vivo, it is difficult to interpret the results when using substrates metabolized by both CYP2C9 and CYP2C8, since there

is a strong linkage disequilibrium between *CYP2C8*3* and *CYP2C9*2*, the latter encoding an enzyme with decreased activity (Yasar et al., 2002). Lundblad et al. (2005) found a significant decrease in arachidonic acid metabolism in individuals carrying the *CYP2C8*3/CYP2C9*2* haplotype when compared with the wild-type individuals; however both CYP2C9 and CYP2C8 contribute to arachidonic acid oxidation whereby the impact of individual genes is impossible to decipher based on this study. Similarly, significant differences for *R*-ibuprofen metabolism were found for *CYP2C8*3/CYP2C9*2* individuals when compared with wild type, but, interestingly, some individuals that carried *CYP2C8*3*, but not *CYP2C9*2*, also had a decreased elimination of *R*-ibuprofen (Garcia-Martin et al., 2004; Martinez et al., 2005). By contrast, Niemi et al. (2003) found that subjects heterozygous for *CYP2C8*3* had an increased metabolism of repaglinide when compared with wild-type and *CYP2C8*4* individuals. Therefore, the in vivo effect of *CYP2C8*3* and the molecular mechanisms underlying CYP2C8 interindividual variability remains uncertain. Recently, 2 haplotypes of *CYP2C8* were identified, one associated with lower and the other with higher activity using both paclitaxel and repaglinide (Rodríguez-Antona et al., in press). The high activity allele carried *CYP2C8*1B*, which binds nuclear factors, while Ile264Met, present in *CYP2C8*4*, was part in some cases of the haplotype with low activity and further genetic analyses are needed to elucidate the mechanism for the low-activity allele. In conclusion, the *CYP2C8* gene is relatively highly conserved, and no important functional variants or null alleles are distributed in the populations. The clinical importance of the *CYP2C8* polymorphism is still unclear, and the different variants remain to be further characterized both in heterologous expression systems and in vivo.

2.4.5. CYP2C9

CYP2C9 is mainly expressed in the liver, at levels that are the highest among the CYP2C enzymes and representing about 20% of the hepatic CYP content. CYP2C9 is involved in the metabolism of ~10% of all drugs, mainly nonsteroidal antiinflammatory drugs (NSAID), oral antidiabetics, antiinfectives, hypnotics, antiepileptics, oral anticoagulants, such as warfarin (Section 3.9), and sulfonylureas (Section 3.11), psychotropics and angiotensin-2 antagonists, among others. In addition, CYP2C9 metabolizes the endogenous substrates arachidonic acid and linolenic acid. There is large interindividual variation in CYP2C9 activity in the population that result in interindividual variations in drug response and also in adverse effects. This variation can be partly caused by environmental factors, such as induction by prototypical CAR, PXR and GR ligands through different elements in the promoter region (Ferguson et al., 2002). In addition, the inhibition of CYP2C9 has to be taken into consideration, as shown by the reduction of CYP2C9 metabolism in healthy women taking oral contraceptives (Sandberg et al., 2004). Concerning gene variations, it is a well known fact that *CYP2C9* polymorphisms have functional consequences for in vitro and in vivo pharmacokinetics and for clinical drug response and side effects. There are more than 30 different SNPs described in the regulatory and coding region of *CYP2C9*, but the polymorphic behavior of CYP2C9 seems to be determined mainly by 2 common coding variants: *CYP2C9*2* (R144C) and *CYP2C9*3* (I359L), both yielding enzymes with decreased activity. *CYP2C9*2* and *CYP2C9*3* are mainly present in Caucasians (11% and 7% frequency, respectively) while the frequencies are lower in Africans. In Asians, *CYP2C9*2* has indeed not been detected (Schwarz, 2003; Kirchheiner & Brockmoller, 2005). In vitro results have shown that the substrate affinity of CYP2C9.2 varies as compared with the wild-type enzyme. Consequently, for some substrates, it is unaffected, but with some other drugs such as acenocoumarol, the activity is severely decreased (Thijssen & Ritzen, 2003). The CYP2C9.3 protein, containing the conservative amino acid substitution I359L, results in a significant reduction of the catalytic activity with only around 10% of the intrinsic clearance of the wild-type enzyme for most CYP2C9 substrates (Takahashi et al., 1998; Thijssen & Ritzen, 2003). *CYP2C9*2* and/or **3* have been shown to affect the oral clearance of at least 17 different CYP2C9 drugs: *S*-acenocoumarol, *S*-phenprocoumon, *S*-warfarin, glimepiride, glyburide, tolbutamide, nateglinide, candesartan, losartan, celecoxib, diclofenac, flubiprofen, *S*-ibuprofen, tenoxicam, fluvastatin, phenytoin and torsemide (Kirchheiner & Brockmoller, 2005), and similarly to the in vitro data, the *CYP2C9*3* allele has stronger pharmacokinetic effects than *CYP2C9*2*. For most substrates, *CYP2C9*3* heterozygous individuals had approximately 50% of the wild type total oral clearance and *CYP2C9*3* homozygous individuals had a 5- to 10-fold reduction. With respect to *CYP2C9*2*, a significant effect was found for *S*-warfarin, acenocoumarol, tolbutamide and celecoxib clearance but not for other drugs (Kirchheiner & Brockmoller, 2005). This suggests differences in substrate specificity among the 3 CYP2C19 enzymes: CYP2C9.1, CYP2C9.2 and CYP2C9.3. On the other hand, the linkage disequilibrium between *CYP2C9*2* and *CYP2C8*3* as discussed above could have an impact on the observed CYP2C9.2 phenotype. In conclusion, the CYP2C9 polymorphism is clinically highly significant and also substrate dependent. This polymorphism has to be taken into account for appropriate dosing in many different therapies.

2.4.6. CYP2C19

Polymorphisms in the *CYP2C19* gene are shown to affect the metabolism of several classes of drugs, including antidepressants and proton pump inhibitors (PPI; for some recent reviews in this topic, see Ingelman-Sundberg, 2004; Wilkinson, 2005; Klotz, 2006). CYP2C19 also appears to play a role in the bioactivation of cyclophosphamide (Timm et al., 2005; Singh et al., 2007). PM carrying 2 defective *CYP2C19* genes are present at a frequency of up to 5% in Caucasian and African populations and approximately 20% in Asians (Desta et al., 2002). *CYP2C19*2* and *CYP2C19*3* are together responsible for the majority of PM alleles, of which *CYP2C19*3* is mainly found in Asians (Xie et al., 2001). The PM genotype and phenotype have proven beneficial in the treatment of gastrointestinal disorders using proton pump inhibitors due to the reduced metabolism of these drugs in such individuals, thus leading to increased drug plasma levels. Therefore, PMs display

an increased healing rate in the treatment of gastric ulcers (Klotz, 2006) and an increased responsiveness to treatment of gastroesophageal reflux disease (Furuta et al., 2002; Kawamura et al., 2003, 2007). The CYP2C19 phenotype also affects the pharmacokinetics of antidepressants; the monoamine oxidase inhibitor (MAOI) moclobemide, the tricyclic antidepressants (TCA) amitriptyline and clomipramine, and the selective serotonin reuptake inhibitors (SSRI) sertraline and citalopram are examples of such drugs (Wang et al., 2001; Yokono et al., 2001; Yu et al., 2001; Shimoda et al., 2002; Herrlin et al., 2003; Yu et al., 2003c). So far, any significant effect of *CYP2C19* genotype on outcome in the treatment of depression has not been shown. In contrast to antiulcer drugs, the dose–effect relationship of antidepressants is much less clear and could thus be an explanation to the low penetrance of *CYP2C19* alleles in antidepressant therapy. The risk for adverse drug reactions has however been associated with *CYP2C19* genotype during amitriptyline drug treatment (Steimer et al., 2005).

The interindividual variation in CYP2C19 activity in vivo among individuals defined as EMs based on genotyping for common defective alleles remains large (Chang et al., 1995; Kim et al., 2002), and this variation could not be explained by the known rare alleles (see www.cypalleles.ki.se). This prompted for investigations of novel *CYP2C19* alleles of importance for this interindividual difference among the EM phenotype group, and we found the *CYP2C19*17* allele causing increased activity due to an increase in CYP2C19 transcription (Fig. 4A; Sim et al., 2006). The allele, which is fairly common in Caucasians and Ethiopians (18%), carries 2 SNPs in the 5′-flanking region, −806C>T and −3402C>T, of which the element carrying −806T was shown to specifically bind hepatic nuclear proteins in electrophoretic mobility shift assays (EMSA). In vivo reporter transfection experiments in mice using 930 bp of 5′-flank revealed an increased rate of transcription compared with *CYP2C19*1*. In 2 different populations, Swedes and Ethiopians, the *CYP2C19*17* allele was found strongly associated with increased CYP2C19 activity in vivo as measured by omeprazole and mephenytoin as probe drugs. A 2- and 4-fold difference in median ratios (omeprazole/5-hydroxyomeprazole and *S*/*R*-mephenytoin, respectively) between *CYP2C19*1* and *CYP2C19*17* homozygotes was observed, respectively. The association is visualized in Fig. 4B, showing the relationship of the *S*/*R*-mephenytoin ratio with the number of *CYP2C19*17* alleles in the Ethiopian subjects. Linear regression of omeprazole metabolic ratio (MR) and area under the plasma concentration–time curve (AUC) in 24 subjects was used to extrapolate MR data to AUC in relation to *CYP2C19*17* in 107 Swedes (Sim et al., 2006), which revealed that the omeprazole AUC in subjects homozygous for *CYP2C19*17* would amount to 60–65% of those homozygous for *CYP2C19*1* after a single oral dose of 20 mg omeprazole. Studies investigating the effect of *CYP2C19*17* on therapeutic outcome are currently at hand, and the initial analysis using 125 ulcer patients on triple therapy with pantoprazole, amoxicillin and metronidazole did not show any significant effect (Kurzawski et al., 2006). In a recent study by Rudberg et al. (2007), they found that patients (n=166) on escitalopram therapy exhibited 42% lower plasma concentrations

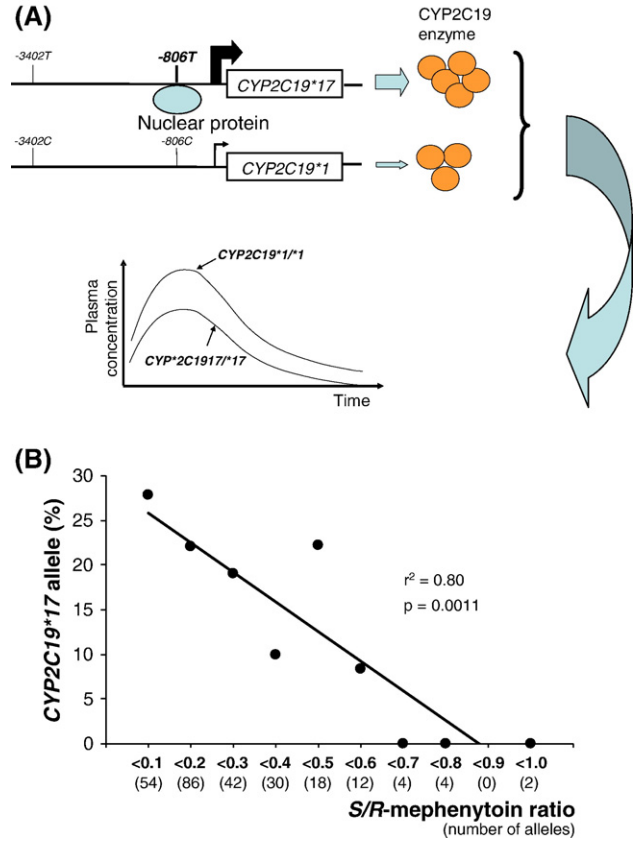


Fig. 4. Increased CYP2C19 expression caused by the *CYP2C19*17* allele. (**A**) The-806C>T mutation in the *CYP2C19*17* allele creates an element similar to a GATA site to, which nuclear proteins are specifically bound. This causes an increased rate of gene transcription leading to an approximately 35–40% lower AUC following treatment with, e.g., antiulcer drugs. Concept from Sim et al. (2006). (**B**) Relationship of *CYP2C19*17* frequency with *S*/*R*-mephenytoin ratio. The number of *CYP2C19*17* alleles is plotted against the *S*/*R*-mephenytoin ratio as a probe for CYP2C19 activity in 126 Ethiopian subjects based on data extracted from Sim et al. (2006). A statistically significant correlation of an increased number of *CYP2C19*17* alleles with increased CYP2C19 activity is apparent.

than those homozygous for *CYP2C19*1* and suggested the necessity of dose adjustment for those carrying *CYP2C19*17*. Further studies in this area are to come.

2.4.7. CYP2D6

CYP2D6 is the most important polymorphic enzyme active in the metabolism of drugs. It is responsible for the metabolism of 25% of all drugs on the market (Evans & Relling, 1999; Eichelbaum et al., 2006), and its polymorphism significantly affects the metabolism of about 50% of these drugs (Ingelman-Sundberg, 2005; for recent reviews, see Kirchheiner et al., 2004a; Zanger et al., 2004; Ingelman-Sundberg, 2005; Bernard et al., 2006). The enzyme is the only one among the drug metabolizing CYPs which is not inducible, and therefore, genetic variation contributes largely to the interindividual variation in enzyme activity. Thus, the polymorphism of the enzyme is of great importance for the metabolism and effects of many drugs, such as antidepressants, neuroleptics, antiarrhytmics, analgesics, antiemetics and anticancer drugs. Currently, more than 63 different functional *CYP2D6* gene variants have been described

(www.cypalleles.ki.se), and these are divided into alleles causing abolished, decreased, normal, and ultrarapid enzyme activity. The most important null alleles are *CYP2D6*4* (splice defect) and *CYP2D6*5* (gene deletion), whereas the common alleles with severely reduced activity are represented by *CYP2D6*10*, *CYP2D6*17* and *CYP2D6*41* (splicing defect). One allele, *CYP2D6*17*, decreases the CYP2D6 activity in a substrate dependent fashion (Wennerholm et al., 2001; Bogni et al., 2005), whereas this has not been shown for other enzyme variants. The *CYP2D6* gene is subject to many CNVs. Initially, alleles with 0, 1, 2, 3, 4, 5 and 13 gene copies were identified (Johansson et al., 1993; Aklillu et al., 1996). The gene duplication events include both functional, partly functional and nonfunctional genes. An investigation by Gaedigk et al. (2007) revealed the following gene duplications events: $*1\times N$, $*2\times N$, $*4\times N$, $*6\times N$, $*10\times N$, $*17\times N$, $*17\times N$[spacer], $*29\times N$, $*35\times N$, $*43\times N$, and $*45\times N$. Many of the novel variant duplications were found in African-Americans. Duplication or multiduplications of active *CYP2D6* genes results in ultrarapid enzyme activity. In contrast to the PM phenotype where a debrisoquine MR antimode of 12.6 can be defined, there is no clear definition for the UM phenotype except for the presence of >2 active gene copies. The EM and the UM phenotype overlap much more than the PM and EM phenotypes (cf. de Leon, 2007).

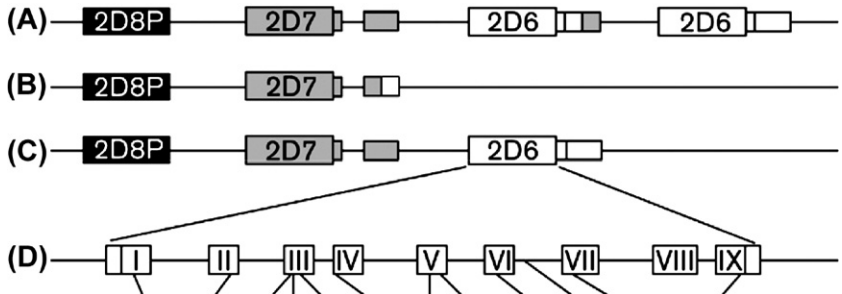
With respect to clinical trials and drug therapy, the allelic constitution of a given patient is an important aspect with respect to proper dosing and for registering a genotype–phenotype correlation among the patients. In Table 2, we try to give a useful estimate regarding the degree of functionality of the various *CYP2D6* alleles based on review of the literature where the effect of the variant alleles on the metabolism of CYP2D6 substrates has been registered (cf. www.cypalleles.ki.se for references). By adding the contribution of the relative activity of the both alleles in a given subject the absolute functionality of CYP2D6 is obtained, thus varying from 0 to 4 and above.

In the past, the PM phenotype has been much discussed in relation to adverse drug reactions. However, also the UM phenotype has been associated to adverse drug reactions, mainly as a result of high levels of the drug metabolite. For example, a recent case of infant death was described to be caused by a breast feeding mother of the UM phenotype and taking high doses of codeine with the resulting formation of morphine at levels being lethal to the infant (Koren et al., 2006). Such aspects have to be considered in drug development where the drug candidate in question is a substrate for CYP2D6. In UMs, up to 10- to-30-fold higher amounts of metabolites can be expected (cf. Dalen et al., 1999), whereby the toxicity of the metabolites has to be taken into serious consideration. In addition, the UM phenotype is one explanatory factor for lack of response of antidepressants (Kawanishi et al., 2004; Rau et al., 2004) and decreased levels of several drugs, which are CYP2D6 substrates, such as tramadol (Stamer et al., 2007), antiemetics (Kaiser et al., 2002), venlafaxine (Shams et al., 2006), morphine (Kirchheiner et al., 2007) and metoprolol (Fux et al., 2005) are evident.

The *CYP2D6* alleles are subject to very important interethnic differences where PMs are mainly found in Europe and UMs in North Africa and Oceania, whereas IMs are to a great extent located in Asia due to the high prevalence of the *CYP2D6*10* allele (Ingelman-Sundberg, 2005). The UM genotype has previously been suggested to have been evolved due to dietary selection pressure in particular in North East Africa (Ingelman-Sundberg, 2005). A recent study by Sistonen et al. (2007) examines the interethnic distribution of different *CYP2D6* alleles in detail. By investigation of 20 different *CYP2D6* haplotypes worldwide (Fig. 5) in 52 populations, they found that the *CYP2D6*1, CYP2D6*2* and *CYP2D6*10* are the most common alleles (Fig. 6). They analyze 12 different mutations whereby they can identify 21 different haplotypes of functional importance (Fig. 5). They also found that the UM phenotype is of higher relevance worldwide as compared with PMs, which however are most common in Europe. The UM phenotype is preferentially distributed in North East Africa and in Oceania (Fig. 7). The absolute highest prevalence for the UM phenotype is found in the Mozabite population in Algeria in North Africa where the allele frequency of *CYP2D6*$*2\times N$ is as high as 28%, indicating that the majority of the population are UMs. Interestingly, 20% of the population in Oceania are also UMs.

Table 2
Functionality of *CYP2D6* alleles on a single chromosome and its related predicted enzyme activity

CYP2D6 allele	Corresponding allele functionality on one of the 2 chromosomes (UM, EM, IM or PM allele)	Corresponding allele functionality on one of the 2 chromosomes expressed as a numeric value	Related enzyme activity/related enzyme functionality
*CYP2D6*1*	EM	1	Normal
*CYP2D6*$1\times N$ where $N\geq 2$	UM	$1\times N$	Increased
*CYP2D6*2*	EM	1	Normal
*CYP2D6*$2\times N$ (N=2,3,4, 5 or 13)	UM	$1\times N$	Increased
*CYP2D6*3*	PM	0	None
*CYP2D6*4*	PM	0	None
*CYP2D6*4×2*	PM	0	None
*CYP2D6*5*	PM	0	None
*CYP2D6*6*	PM	0	None
*CYP2D6*7*	PM	0	None
*CYP2D6*8*	PM	0	None
*CYP2D6*9*	IM	0.7	Decreased
*CYP2D6*10*	IM	0.2	Decreased
*CYP2D6*$10\times N$	IM	$0.2\times N$	Decreased
*CYP2D6*14*	PM	0	None
*CYP2D6*17*	IM	0.5	Decreased
*CYP2D6*$17\times N$	EM (if N=2)	$0.5\times N$	Normal (if N=2)
*CYP2D6*18*	PM	0	None
*CYP2D6*21*	PM	0	None
*CYP2D6*29*	IM	0.7	Decreased
*CYP2D6*35*	EM	1	Normal
*CYP2D6*35×2*	UM	2	Increased
*CYP2D6*36*	IM	0.05	Decreased
*CYP2D6*36_*10*	IM	0.25	Decreased
*CYP2D6*36×2*	IM	0.1	Decreased
*CYP2D6*41*	IM	0.5	Decreased
*CYP2D6*41×2*	EM	1	Normal
*CYP2D6*44*	PM	0	None



Haplotype	100	1023	1659	1661	1707	1846	2549	2613-15	2850	2988	3183	4180	Dup	Enzyme activity
*1	C	C	G	G	T	G	A	AGA	C	G	G	G	–	norm
*2	C	C	G	C	T	G	A	AGA	T	G	G	C	–	norm
*3	C	C	G	G	T	G	–	AGA	C	G	G	G	–	none
*4	T	C	G	C	T	A	A	AGA	C	G	G	C	–	none
*4B	T	C	G	G	T	A	A	AGA	C	G	G	C	–	none
*5	–	–	–	–	–	–	–	–	–	–	–	–	–	none
*6	C	C	G	G	–	G	A	AGA	C	G	G	G	–	none
*9	C	C	G	G	T	G	A	–	C	G	G	G	–	decr
*10	T	C	G	C	T	G	A	AGA	C	G	G	C	–	decr
*17	C	T	G	C	T	G	A	AGA	T	G	G	C	–	decr
*29	C	C	A	C	T	G	A	AGA	T	G	A	C	–	decr
*39	C	C	G	C	T	G	A	AGA	C	G	G	C	–	norm
*41	C	C	G	C	T	G	A	AGA	T	A	G	C	–	decr
*1661	C	C	G	C	T	G	A	AGA	C	G	G	G	–	norm
*4180	C	C	G	G	T	G	A	AGA	C	G	G	C	–	norm
*1xN	C	C	G	G	T	G	A	AGA	C	G	G	G	+	incr
*2xN	C	C	G	C	T	G	A	AGA	T	G	G	C	+	incr
*4xN	T	C	G	C	T	A	A	AGA	C	G	G	C	+	none
*10xN	T	C	G	C	T	G	A	AGA	C	G	G	C	+	decr
*41xN	C	C	G	C	T	G	A	AGA	T	A	G	C	+	decr
*1661xN	C	C	G	C	T	G	A	AGA	C	G	G	G	+	incr

Fig. 5. Determination of 20 different *CYP2D6* haplotypes by SNP analysis. By analyzing only 12 different SNPs in the *CYP2D6* gene, 20 different haplotypes can be determined. Data from Sistonen et al. (2007) are reproduced with permission from the authors and from the publisher.

However, the duplication event in the Oceanian population appears to be of another origin since the allele found is *CYP2D6**1 × *N* and has most probably evolved independently from the duplication selected for in North Africa.

We continuously get new submissions to the CYP allele Web page concerning new variants of the *CYP2D6* gene. In total, more than 100 variant alleles are now present on the CYP2D6 Web page (www.cypalleles.ki.se/cyp2d6). The continuous expansion of the number of allelic variants makes a complete prediction of the CYP2D6 phenotype by genotyping impossible. One might estimate that genotyping for 12 SNPs representing 20 different haplotypes as presented by Sistonen et al. (2007) would predict the real phenotype by about 90–95% accuracy. During drug development and for retrospective aspects of drug treatment one has to bear this in mind. Therapeutic drug monitoring (TDM) or analysis of the phenotype by administering a probe drug-like debrisoquine or dextromethorphan (Frank et al., 2007; Fuhr et al., 2007) might provide a more accurate estimate of the CYP2D6 phenotype. Besides the detection of specific *CYP2D6* alleles mainly having functional mutations in the open reading frame, one must also consider the possibility for the presence of many different haplotypes carrying still unidentified mutations. This is very plausible, since for example the endoxifen levels (an antiestrogen that is activated from tamoxifen by CYP2D6) among EM subjects vary greatly (Borges et al., 2006), and a similar wide distribution of phenotypes with respect to sparteine and dextromethorphan has also previously been shown by Luo et al. (2005). One can therefore not exclude the presence of phenotypically important unidentified common *CYP2D6* variants. Furthermore mutations in CYP2D6 can give rise to altered substrate specificity as shown, e.g., by Shen et al. (2007).

In conclusion, the polymorphism of *CYP2D6* appears to be the most clinically relevant of all presently known polymorphic genes with respect to drug therapy. Predictive or retrospective genotyping can explain many cases of non response or adverse drug reactions in patients treated with CYP2D6 substrates. In our opinion, the clinical practice would benefit from this information, which could be implemented already today. In the future, this problem will however decrease in magnitude since the drug industry tries to avoid the development of drugs which are pharmacokinetically influenced to a great extent by the *CYP2D6* polymorphism.

2.4.8. CYP3A4/5/7

2.4.8.1. General aspects. The CYP3A drug-metabolizing enzymes facilitate the metabolism and elimination of a wide range of structurally different xenobiotics and of 50% of all therapeutic drugs used in the clinics (Bertz & Granneman, 1997). In addition, they participate in the metabolism of key endogenous substrates, such as retinoic acid, steroid hormones and bile acids. There are 4 *CYP3A* human genes, *CYP3A4*, *CYP3A5, CYP3A7* and *CYP3A43*, and 3 pseudogenes, *CYP3AP1*, *CYP3AP2* and *CYP3AP3*, all located in one locus at chromosome 7q21-q22.1 (Finta & Zaphiropoulos, 2000; Gellner et al., 2001). CYP3A43 most likely does not translate into active enzyme (Westlind et al.,

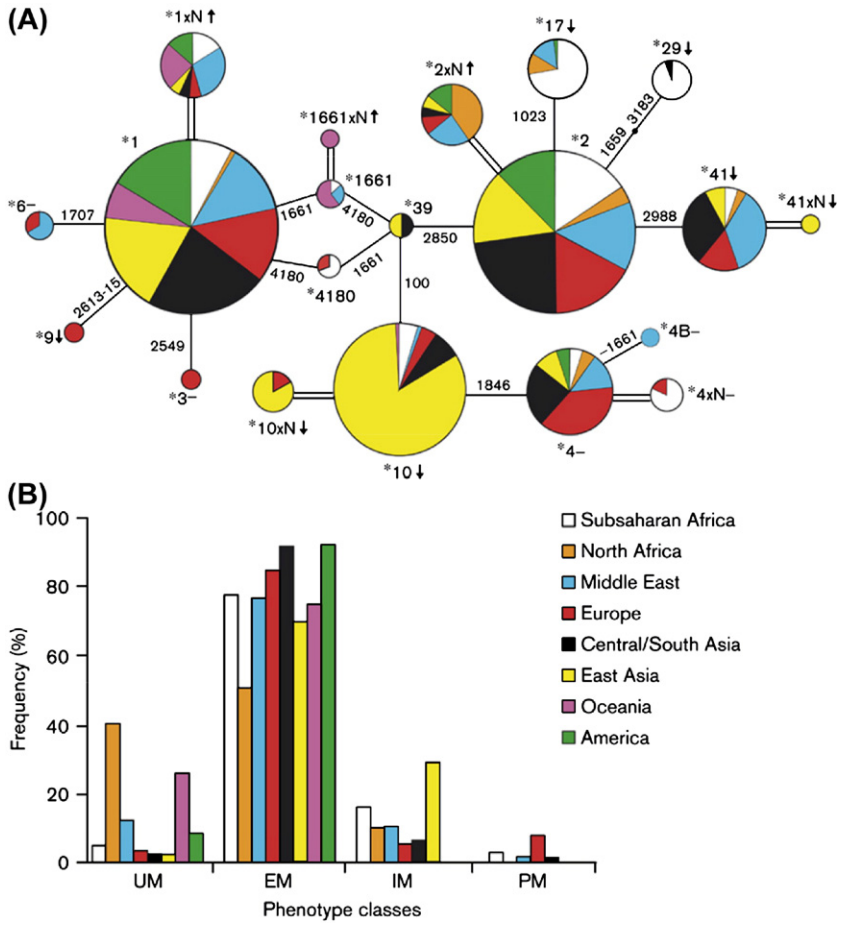


Fig. 6. Global distribution of *CYP2D6* alleles and phenotypes. The globally most common variant *CYP2D6* alleles are *CYP2D6*1*, *CYP2D6*2* and *CYP2D6*10*. The UM phenotype is globally the most important variant phenotype, whereas PMs are preferentially localized in Europe. Figure from Sistonen et al. (2007) is reproduced with permission from the authors and the publisher.

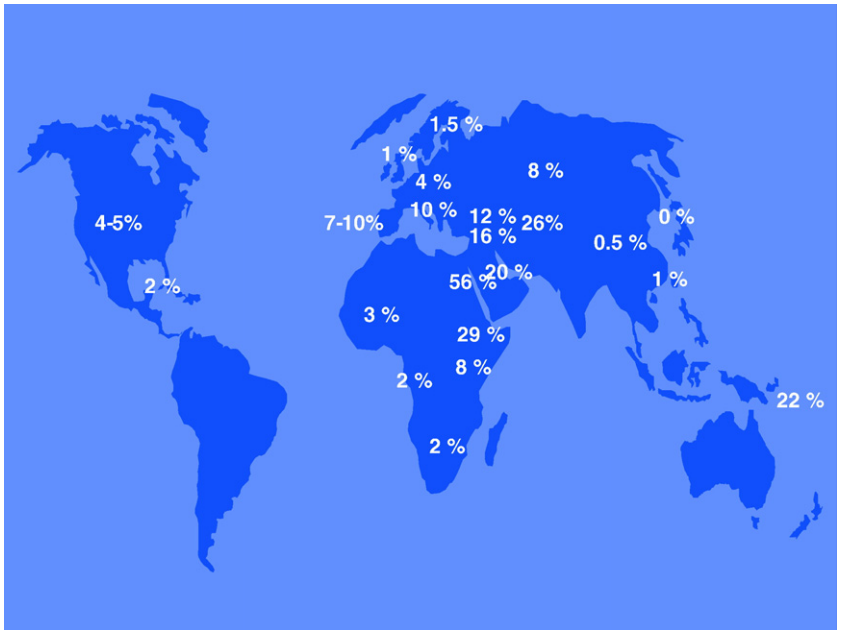


Fig. 7. Global distribution of individuals carrying duplication of the *CYP2D6*1* or *CYP2D6*2* genes presented as percentage of the population carrying such alleles. The *CYP2D6* gene duplications appears to have been selected for in 2 different regions of the world, in North East Africa where the *CYP2D6*2×N* genotype is very common and in Oceania where duplication of the *CYP2D6*1* gene has occurred. Data mainly from Ingelman-Sundberg (2001, 2005) and Sistonen et al. (2007).

2001). The clinically relevant CYP3A enzymes, *CYP3A4*, *CYP3A5* and *CYP3A7*, have a predominant hepatic expression and similar substrate specificities, hampering the assessment of their individual contributions to drug metabolism (Williams et al., 2002). In the case of CYP3A4 and CYP3A7, they show an opposite expression pattern during development, and in general, it is considered that CYP3A4 contribution to fetal CYP3A metabolism is null, while adult CYP3A4 metabolism is much higher than that of CYP3A7 (Lacroix et al., 1997). On the other hand, *CYP3A5* is transcribed in the liver at similar levels at all developmental stages, although being polymorphically expressed. Therefore, the fetal hepatic CYP3A activity corresponds to CYP3A7 and CYP3A5, while that of adult CYP3A activity has been considered mainly to correspond to CYP3A4 and CYP3A5 (Stevens et al., 2003).

The CYP3A adult phenotype has been assessed using several substrates (e.g., midazolam, erythromycin, quinine), revealing that a moderate 4- to 6-fold interindividual variation represents most of the population (Lin et al., 2002; Floyd et al., 2003; Rodriguez-Antona et al., 2005c). Since the expression of CYP3A4 and CYP3A5 can be induced by PXR, CAR and GR ligands, both environmental and genetic factors can influence the CYP3A activity; however, the genetic contribution has been estimated to be larger (Ozdemir et al., 2000), suggesting that the polymorphisms in *CYP3A4* and/or *CYP3A5* could predict CYP3A phenotype. In this respect, *CYP3A5* is a highly polymorphic enzyme as a consequence of mutations that severely diminish the synthesis of functional CYP3A5 protein (*CYP3A5*3*, **6* and **7*; Hustert et al., 2001; Kuehl et al., 2001). *CYP3A5*3* is the most common defective allele with an allele frequency of about 90%, 75% and 20% in Caucasians, Asians and Africans, respectively, whereas *CYP3A5*6* and *CYP3A5*7* are not present in Caucasians and Asians and have a 17% and 8% frequency in Africans, respectively (Lee et al., 2003). The underlying reason for the evolvement of *CYP3A5* as polymorphic in nature could be its relatively small contribution to the total CYP3A hepatic metabolism, due to the higher protein content of CYP3A7 and CYP3A4 in fetal and adult liver, respectively (Lacroix et al., 1997; Tateishi et al., 1997; Stevens et al., 2003; Westlind-Johnsson et al., 2003). This is supported by the modest impact of the *CYP3A5* null variants on total CYP3A metabolism as measured by probes such as midazolam and quinine (Floyd et al., 2003; Mirghani et al., 2006). Because of the much higher frequency of *CYP3A5*1* in Africans, as compared with Caucasians, the impact of CYP3A5 is however more significant in, e.g., Tanzanians as recently shown using the drug saquinavir (Josephson et al., 2007).

In contrast to *CYP3A5*, the important role in the metabolism of endogenous compounds likely imposed limitations to the variation in *CYP3A4* and resulted in rare deleterious alleles. Extensive studies searching allelic variants in the coding regions of *CYP3A4* have been carried, and up until today, 20 different CYP3A4 variant proteins have been described, some of them representing proteins of decreased activity (i.e., **6*, **17*, **20*). However, their low frequency rule them out as causation for the common interindividual differences in CYP3A4 activity and suggests that the genetic variation responsible for the variability must be located elsewhere in the *CYP3A4* gene or in genes acting in *trans*. Interestingly, a parallel variation between *CYP3A4* mRNA and hnRNA contents in human liver samples suggests that differences in *CYP3A4* transcription rate are the cause for the interindividual variability (Hirota et al., 2004; Rodriguez-Antona et al., 2005c). In agreement with this, we recently showed in Tanzanian healthy volunteers using quinine as probe that a nucleotide change in the promoter region, *CYP3A4*1B* (−392A>G), was associated to a significantly lower activity than *CYP3A4*1A*, due to a diminished binding of nuclear proteins to the proximal promoter of *CYP3A4* (Rodriguez-Antona et al., 2005c). Thus, *CYP3A4*1B* explains, to some extent, the genetic basis for the interindividual variation in CYP3A4 expression, but other unknown genetic factors must be of significance. In agreement, it has been shown that there is an allelic expression imbalance of *CYP3A4* mRNA and that the allelic expression ratio correlated with the total *CYP3A4* mRNA level. In this way, the samples with a large difference of transcript level between the 2 alleles had low levels of total hepatic CYP3A4 mRNA and low metabolic capability as assessed by testosterone 6β-hydroxylation (Hirota et al., 2004). Thus, expression imbalance seems to play an important role in CYP3A4 expression variability. However, the mechanism that generates the allelic imbalance remains unknown.

CYP3A7 is a predominantly fetal enzyme, with its expression starting after 50–60 days of gestation and continuing up to 6 months of postnatal age (Lacroix et al., 1997; Stevens et al., 2003). In the fetus, CYP3A7 plays an important role in the metabolism of endogenous substrates, such as key steroids and retinoic acid, and also in the metabolism of xenobiotics reaching the fetus from the maternal circulation. Interindividual variation in CYP3A7 expression could therefore result in interindividual differences in embryotoxicity and teratogenicity. Similarly to CYP3A4, CYP3A7 is a well-conserved gene, and up to today only one frameshift mutation (*CYP3A7*3*, Shin et al., in preparation; see www.cypalleles.ki.se) and one coding polymorphism have been described (*CYP3A7*2*; T409R). The *CYP3A7*2* SNP has an allele frequency of 8%, 28% and 62% in Caucasians, Asians and Africans, respectively, and encodes an enzyme with a moderately higher activity than CYP3A7.1 (Rodriguez-Antona et al., 2005b). Recent work by Leeder et al. (2005) using fetal liver microsomes found no significant differences in dehydroepiandrosterone (DHEA) metabolism between livers carrying *CYP3A7*2* or *CYP3A7*1*, but at the moment, the impact of *CYP3A7*2* on fetal drug clearance and endogenous substrates metabolism is still unclear. An additional functional CYP3A7 enzyme, CYP3A7.1L, has recently been described. This enzyme results from a mRNA containing 13 exons of *CYP3A7*, the last one lacking the stop codon, and 2 additional exons from *CYP3AP1* at the 3′ end, formed by the splicing between *CYP3A7* and the pseudogene *CYP3AP1* (Finta & Zaphiropoulos, 2000; Rodriguez-Antona et al., 2005a). This enzyme has a different expression pattern than CYP3A7 and is highly polymorphic as the result of a SNP near the splicing site of exon 14. In addition to polymorphisms resulting in alternative CYP3A7 proteins, a genetic variation in *CYP3A7* promoter *CYP3A7*1C* has been shown to be relevant for

CYP3A7 expression (Kuehl et al., 2001; Burk et al., 2002; Sim et al., 2005). In *CYP3A7*1C*, the PXR element in the proximal promoter is replaced by the corresponding region of *CYP3A4*, which can result in a relevant *CYP3A7* expression in adult liver (Figs. 8 and 9). Development of CYP3A7-specific antibodies allowed us to conclude that about 10% of adult livers express significant CYP3A7 protein levels (24–90 pmol/mg) that contribute on average to 24% of total CYP3A protein in these livers, and which was partially explained by the presence of the *CYP3A7*1C* allele (Sim et al., 2005). It is noteworthy that this quantification suggests that CYP3A7 is expressed at a similar or even higher level than the polymorphically expressed CYP3A5, which contributes to 16% of total CYP3A in livers expressing CYP3A5 (about 10%) (Westlind-Johnsson et al., 2003). The impact of this polymorphism on general adult CYP3A drug metabolism activity is not expected to be influential due to the still predominant expression of CYP3A4 (Sim et al., 2005). However, the impact of *CYP3A7*1C* on the plasma levels of DHEA and DHEA sulfate (DHEAS) could be relevant due to the high specificity of CYP3A7 for these substrates, although with a 4-fold preference of DHEA compared with DHEAS (Torimoto et al., 2006). Indeed, one study has found an association of the CYP3A7 high-expression phenotype conferred by the *CYP3A7*1C* allele with decreased endogenous levels of DHEAS in 2 separate populations of elderly subjects (n=208, women and men; n=345, men; Smit et al., 2005). Based on the plausible association of reduced DHEAS with osteoporosis, Bacsi et al. (2007) investigated the effect of the *CYP3A7*1C* allele on DHEAS serum levels and bone density in 217 and 102 postmenopausal women, with and without osteoporosis, respectively. An association of *CYP3A7*1C* and decreased bone density was found, whereas there was no relationship of the allele with serum DHEAS levels and an effect of *CYP3A7* genotype on bone density irrespective of DHEAS levels was suggested. It is apparent that the effect of *CYP3A7* genotype on DHEAS and osteoporosis needs further attention in order to decipher the effect of CYP3A7 on the independent variables and the possible connection between them.

CYP3A7 has been shown to efficiently metabolize all-*trans* retinoic acid (atRA) to 4-hydroxy RA at a rate of almost 3 times higher than that of CYP3A5 and 15 times higher than that of CYP3A4 (Marill et al., 2000). atRA is also metabolized to its 4-oxo, 18-hydroxy and 5,6-epoxy derivatives by the contribution of several different P450s, including CYP3A7, but at significantly lower efficiency than the 4-hydroxylation carried out by CYP3A7 (Marill et al., 2000). atRA (Tretinoin) is used to treat acute promyelocytic leukemia (APL) and can alone induce a very high frequency of hematological remission, but is however normally used together with chemotherapy to induce stable transmission (Lengfelder et al., 2005). It can be hypothesized that an increased metabolism of atRA mediated by a CYP3A7 high-expression phenotype would impact on the outcome of APL treatment due to the efficient metabolism of atRA by the CYP3A7 enzyme. The 4-hydroxylation of the 9-*cis* and 13-*cis* RAs is also carried out by CYP3A7, of which the 13-*cis* hydroxylation is mainly catalyzed by CYP3A7 whereas the 9-*cis* hydroxylation is more dependent on CYP2C8 and CYP2C9 (Marill et al., 2002). 13-*cis* RA (Isotretinoin) is an effective medication for the treatment of acne. Since adverse effects are significant for this type of acne therapy, individualized dosing based on drug effectiveness and side effects is often applied (Cooper, 2003; van de Kerkhof et al., 2006), and thus high expression CYP3A7 phenotypes are not expected to affect treatment outcome. 9-*cis* RA (Alitretinoin) is used in the treatment of AIDS-related Kaposi's sarcoma (Cheer & Foster, 2000). However, due to the topical administration of Alitretinoin and also the significant involvement of CYP2C enzymes in its metabolism, adult CYP3A7 expression is not expected to be of any relevance for this drug.

In Fig. 8, a summary on the contribution of the different CYP3A enzymes to total CYP3A in an average adult human liver is presented based on the data by Westlind-Johnsson et al. (2003) and Sim et al. (2005) using peptide-specific antibodies and peptide conjugates as standards. The respective levels of CYP3A4, CYP3A5 and CYP3A7 in an average Caucasian liver are 173, 5 and 4 pmol/mg, respectively, whereas CYP3A43 is not expressed. These amounts equals to an average contribution of CYP3A5 and CYP3A7 to 2–3% to total CYP3A levels, with the remaining levels being composed of CYP3A4. If one assumes a liver with average CYP3A4 expression and the highest levels of CYP3A5 and CYP3A7 reported in these studies, CYP3A5 and CYP3A7 would at maximum contribute to 20–30% to total CYP3A levels.

2.4.8.2. The CYP3A locus. Importantly, there is a high degree of linkage disequilibrium between *CYP3A4, CYP3A5* and *CYP3A7* in Asians and Caucasians. However, the African *CYP3A* locus is segmented in 4 blocks of high linkage disequilibrium spanning the *CYP3A4* promoter, the *CYP3A4*

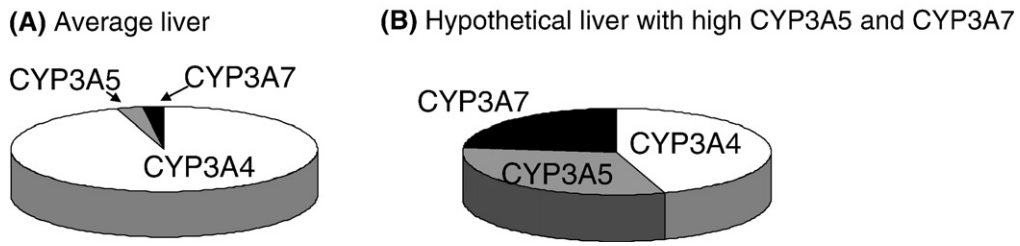
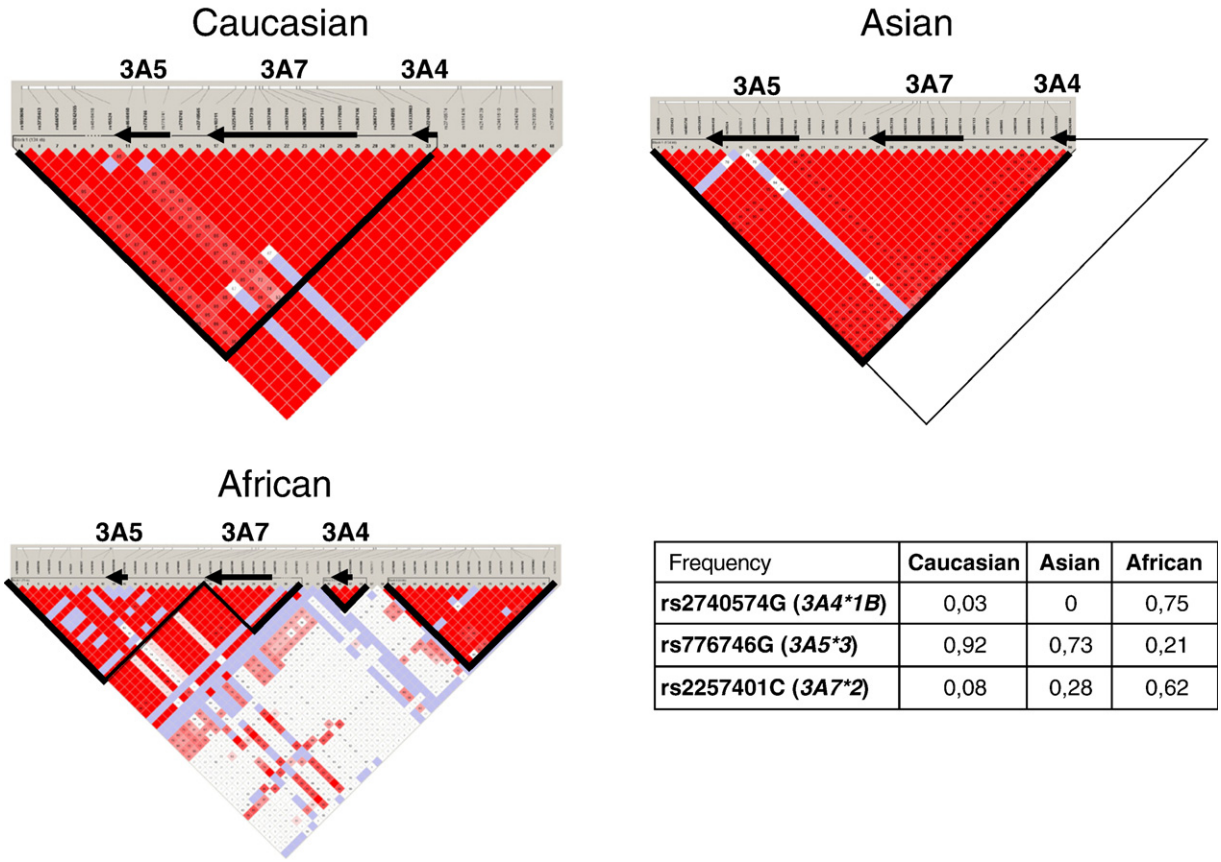


Fig. 8. Contribution of different CYP3A isoforms to total CYP3A levels in Caucasian livers. (**A**) Amount of CYP3A4, CYP3A5 and CYP3A7 in an average adult Caucasian human liver. (**B**) Illustration of the theoretical maximum amounts of CYP3A5 and CYP3A7 in a liver with average CYP3A4 expression. The results are based on data presented in Sim et al. (2006) and Westlind-Johnsson et al. (2003).

gene, including the 3′-UTR, *CYP3A7* and a region containing *CYP3A5* (Figs. 9 and 10). In Caucasians and Asians, the most common *CYP3A* haplotype includes *CYP3A4*1A*, *CYP3A7*1*, *CYP3A7_39256T* and *CYP3A5*3*. In fetal liver, this haplotype is associated to a phenotype that result in the expression of CYP3A7.1 and CYP3A7.1L, while in adult liver, only CYP3A4 protein is expressed. The minor haplotypes in Caucasians and Asians include *CYP3A7*2, CYP3A7_39256A* and *CYP3A5*1* and results in the expression of CYP3A7.2 and CYP3A5 in fetal liver and CYP3A4 and CYP3A5 in adult liver, whereby it seems to be associated to a high detoxification capacity. Concerning *CYP3A4*1B*, this allele, which is in linkage disequilibrium in Caucasians with *CYP3A5*1*, is not present in Asians. Because *CYP3A4*1B* results in a decreased transcription (Rodriguez-Antona et al., 2005c), *CYP3A5*1* could compensate for this allele, resulting in similar total CYP3A activity as present in *CYP3A4*1A*/*CYP3A5*3* adult livers. African *CYP3A* haplotypes are diverse and include combinations of all of the functional *CYP3A* SNPs discussed here.

When the different haplotypes are studied with respect to the distribution in various ethnic groups, it is evident that in Caucasians and Asians one haplotype that includes *CYP3A4*1A*, *CYP3A7*1, CYP3A7_39256T* and *CYP3A5*3* was driven to a near-fixation frequency ($q=0.91$ and $q=0.72$, respectively; Thompson et al., 2004; Rodriguez-Antona et al., 2005c). This data suggests that these SNPs, or other(s) SNPs that are in linkage disequilibrium and have the possibility to cause a functional change in CYP3A, must have provided a significant adaptation advantage and contributed to the complex evolutionary history of this genomic region. With respect to CYP3A5, *CYP3A5*3*, *CYP3A5*6* and *CYP3A5*7* have an similar phenotype (lack or very low funtional CYP3A5 protein), making it difficult to understand why only *CYP3A5*3* was selected to a high frequency in Asians and Caucasians. In fact in Africans, there are 4 haplotypes with similar frequencies that have the same CYP3A5 phenotype (haplotypes 1, 6, 8, and 9 in Fig. 10), but only one of these, the one with the lowest frequency, was selected for. With respect to CYP3A7, the haplotype with highest frequency in Asians and Caucasians is the one corresponding to *CYP3A7*1/ CYP3A7_39256T.* Three haplotypes containing these SNPs are found in Africans: 2 together with *CYP3A5*3* and 1 with *CYP3A5*1* (haplotypes 1, 7, and 8 in Fig. 10), again present with similar frequencies. With respect to CYP3A4, the most frequent haplotype present in Caucasians and the only one present in Asians contain *CYP3A4*1A.* In Africans,



Frequency	Caucasian	Asian	African
rs2740574G (*3A4*1B*)	0,03	0	0,75
rs776746G (*3A5*3*)	0,92	0,73	0,21
rs2257401C (*3A7*2*)	0,08	0,28	0,62

Fig. 9. Interethnic differences in the *CYP3A* locus. 200 kb of the *CYP3A* locus, including *CYP3A5*, *CYP3A7* and *CYP3A4* is represented for different populations studied in the HapMap project: Caucasian , Asian and African. One single block of linkage disequilibrium could be identified for Europeans and Asians, whereas notably, for the Africans, 4 blocks could be identified. The white section in the Asian locus represents data that is not available. The frequency of 3 functional SNPs in the *CYP3A* locus, rs2740574A>G (*CYP3A4*1B*), rs776746A>G (*CYP3A5*3*) and rs2257401G>C (*CYP3A7*2*), is shown for the 3 different populations in the insert table, illustrating the large interethnic differences in the distribution of the alleles. Data from www.HapMap.org.

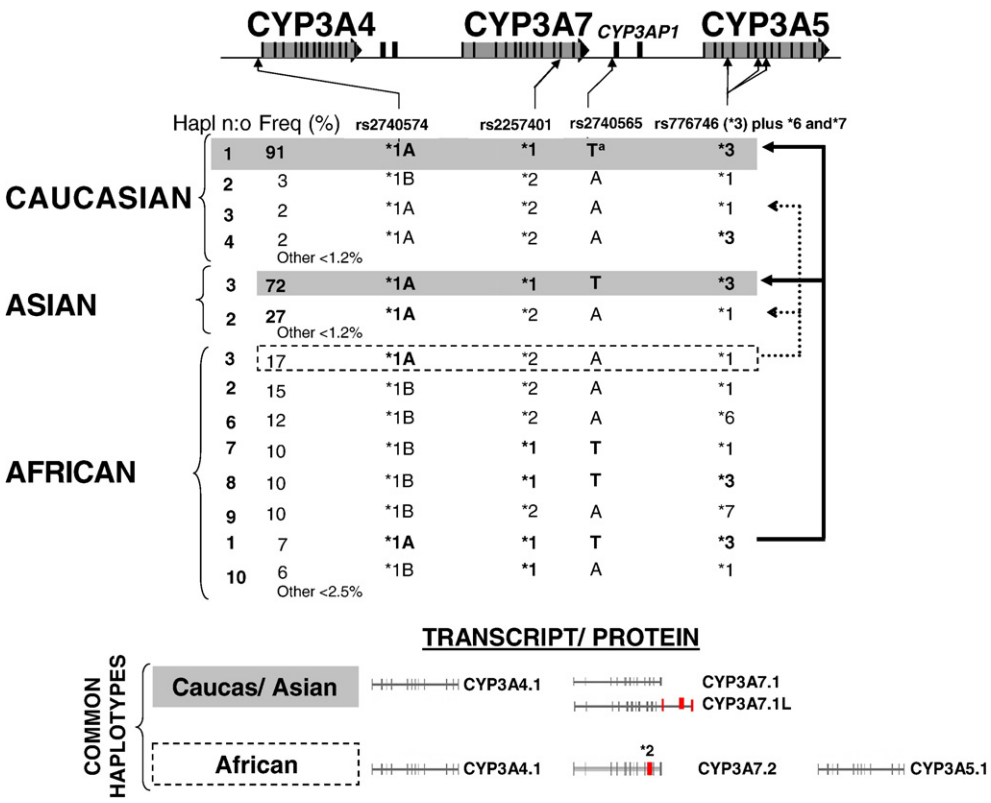


Fig. 10. Frequency of the different *CYP3A* haplotypes and resulting phenotypes. The structure of the *CYP3A* locus in chromosome 7 is shown in the upper part: grey rectangles represent genes with the 13 exons represented as black lines, black rectangles represent the exons of the pseudogenes. The arrows indicate the position of the polymorphisms corresponding to *CYP3A4*1B, CYP3A7*2, CYP3A7_39256T>A, CYP3A5*3, CYP3A5*6* and *CYP3A5*7.* Haplotypes were inferred using genotype data from unrelated individuals: 86 Tanzanians, 96 Chinese, and a mix of 93 individuals of different European ancestries (majority being Spanish) and 186 and Swedes, representing the Caucasian population (Rodriguez-Antona et al., 2005b). *CYP3A5*6* and *CYP3A5*7* were assumed not to be present in Caucasians and Asians. The frequency of the inferred haplotypes is expressed as percentage. Lines with arrow ends indicate the haplotype that was selected in Asians and Caucasians. The most frequent haplotype in Caucasians and Asians is highlighted in grey, while the most frequent African haplotype is in a dashed rectangle. The CYP3A transcripts and proteins resulting from these 2 haplotypes are shown in the bottom.

there are only 2 haplotypes containing *CYP3A4*1A* (1 and 3 in Fig. 10), both present in Asians. This could suggest that *CYP3A4*1A* might have been selected for during the evolution of the *CYP3A* locus (Rodriguez-Antona et al., 2005c). However, other SNP(s) in linkage disequilibrium with *CYP3A4*1A* could have been the leading force for the selection.

3. Therapies

The most important aspect of CYP polymorphism is to what extent it affects the outcome of clinical drug treatment. In this section, we try to emphasize some relevant therapeutic areas where CYP polymorphism significantly influences the response of drugs or the incidence of adverse drug reactions.

3.1. Cancer

Pharmacogenetics has a great relevance in cancer therapy because chemotherapeutic agents are, in general, unspecific and with narrow therapeutic indexes that result in frequent severe or even fatal toxicities. In addition, a lack of therapeutic effect can have critical consequences for the patient (Rodriguez-Antona & Ingelman-Sundberg, 2006). In this way, the activation of cyclophosphamide to 4-hydroxycyclophosphamide (Huang et al., 2000), which is mediated by CYP2B6, CYP3A4, CYP2C19 and CYP2C9 could be affected by polymorphisms in the corresponding genes. This was confirmed by Timm et al. (2005) in a study with 60 cancer patients on cyclophosphamide treatment, showing that individuals carrying the inactive *CYP2C19*2* allele had a significant decreased cyclophosphamide elimination. Similarly, Takada et al. (2004) found that in pulse cyclophosphamide treatment of proliferative lupus nephritis, heterozygous or homozygous *CYP2C19*2* patients had a significantly lower risk of developing premature ovarian failure and that subjects homozygous for *CYP2C19*2* had a higher probability of poor renal response. Homozygous *CYP2B6*5* patients had a significantly higher probability of reaching end-stage renal disease with double serum creatine levels (Takada et al., 2004). Tegafur is also an anticancer prodrug, which is activated to 5-fluorouracil by CYP2A6. Regarding this drug, a patient with a poor tegafur metabolizing phenotype was heterozygous for *CYP2A6*4* and *CYP2A6*11*, suggesting that *CYP2A6* polymorphisms impact tegafur treatment (Daigo et al., 2002). Additionally, combined genotypes of *CYP3A4, CYP3A5*, glutathione *S*-transferases (*GST*) *M1* and *GSTT1* influenced the probability of

treatment failure after high-dose adjuvant chemotherapy for node-positive breast cancer patients who received anthracycline-based adjuvant chemotherapy followed by high-dose multiagent chemotherapy with stem-cell rescue (DeMichele et al., 2005). Patients homozygous for *CYP3A4*1B* and *CYP3A5*3* who did not carry homozygous deletions in neither *GSTM1* nor *GSTT1* (low-drug genotype group) had an almost 5-fold poorer disease-free survival and a 4-fold poorer overall survival compared with women who did not carry any *CYP3A4*1B* or *CYP3A5*3* variants but had deletions in both *GSTT1* and *GSTM1* (high-drug genotype group; DeMichele et al., 2005).

With respect to anticancer drugs of low toxicity, tamoxifen is an estrogen receptor modulator widely used in breast cancer treatment and metabolized by CYP2D6 to the key metabolites 4-hydroxytamoxifen and endoxifen (Jin et al., 2005). Endoxifen has about 100 times greater affinity for the estrogen receptor than tamoxifen (Jordan et al., 1977; Clarke et al., 2003; Jin et al., 2005) and tamoxifen-treated women homozygous for the defective allele *CYP2D6*4* have been shown to have a worsened relapse-free time and disease-free survival rate, in addition to not experiencing hot flashes at the same magnitude as compared with women heterozygous or homozygous for the wild-type allele (Goetz et al., 2005, 2006). In a subsequent study, it was estimated that CYP2D6 PM women had the highest risk of breast cancer relapse (HR 3.12, $p = .007$) and that CYP2D6 metabolism, as measured by genetic variation and enzyme inhibition, was found to be an independent predictor of breast cancer outcome in postmenopausal women receiving tamoxifen for early breast cancer (Goetz et al., 2007). Medications which decrease CYP2D6 activity such as antidepressants, should be taken into account when prescribing tamoxifen and it appears that co-medication in certain cases should even be avoided. Thus, the determination of *CYP2D6* genotype may be of value in selecting adjuvant hormonal therapy. Prospective adjuvant studies are required to determine the best therapy (tamoxifen or aromatase inhibitors) for patients with different CYP2D6 metabolism.

The *CYP2D6* genotype is also relevant for cancer patients with respect to the action of the antiemetic drugs such as the 5-HT3 receptor antagonists tropisetron and ondansetron. Lower plasma levels and higher frequency and intensity of vomiting was found in subjects carrying a higher number of active *CYP2D6* gene copies (Kaiser et al., 2002).

Antiemetics are usually used during cancer treatment and several studies by in particular Brockmöller and collaborators indicate a very high influence of the CYP2D6 polymorphism (Tremblay et al., 2003; Candiotti et al., 2005; Ho & Gan, 2006). In particular, the UM phenotype makes the effect of the treatment much less effective, and a dose adjustment for these kind of drugs should indeed be considered.

3.2. Depression

Depression pharmacogenetics is mostly important with respect to the genetic polymorphism of CYP2D6. This area has been covered relatively recently by Kirchheiner et al. (2004a) and dosage recommendations in relationship to CYP2D6 genotypes have been presented (Kirchheiner et al., 2004a; Thuerauf & Lunkenheimer, 2006). In general, TCAs are better substrates for CYP2D6 than any other CYP, thus making CYP2D6 polymorphism very penetrant for the clinical outcome. This is also why therapeutic drug monitoring of all tricyclics and venlafaxine are strongly recommended by a European consortium (Baumann et al., 2004) in order to investigate the concentration–effect relationships.

CYP2C19 is also involved in the metabolism of several antidepressants. The influence of CYP2C19 activity on the pharmacokinetics and clinical effect of antidepressant drugs is likely dependent on the relative contribution of CYP2C19 metabolism compared with mainly other CYPs (particularly CYP2D6) and the outcome of CYP2C19 metabolism (pharmacologically active or inactive metabolites). For moclobemide, a MAOI, a 3-fold higher AUC, has been observed in CYP2C19 PM compared with EM subjects after a single oral dose (Yu et al., 2001), and the CYP2C19 probe drug omeprazole and moclobemide inhibit each other's metabolism in CYP2C19 EMs (Yu et al., 2001; Cho et al., 2002). The plasma concentration of moclobemide has not been associated with therapeutic efficacy, instead there appears to be a correlation of plasma concentration with side effects (Bonnet, 2003), whereby an increased risk of side effects could be expected in CYP2C19 PMs. Amitriptyline, a TCA, is demethylated to nortriptyline by CYP2C19. The *CYP2C19* genotype affects the MR of amitriptyline to nortriptyline, as well as the AUC of them both (Jiang et al., 2002; Shimoda et al., 2002; van der Weide et al., 2005). However, since the sum of amitriptyline and nortriptyline levels are used in therapeutic drug monitoring (Perry et al., 1994), CYP2C19 activity would not be expected to affect treatment response and accordingly no relationship between *CYP2C19* genotype and response has been detected (Steimer et al., 2005). However, CYP2C19 can affect the risk of adverse events, since nortriptyline levels correlate with side effects, as does also the number of active *CYP2C19* genes (Steimer et al., 2005). Specifically, a combination of high CYP2C19 activity and low CYP2D6 activity confers the highest risk of side effects since CYP2C19 produces the active metabolite nortriptyline, whereas CYP2D6 metabolizes nortriptyline into the inactive metabolite 4-hydroxynortriptyline (Steimer et al., 2005).

The steady-state plasma concentration of the TCA clomipramine has been shown to be affected by the *CYP2C19* genotype; PMs have a 76% and 41% higher dose- and weight-adjusted plasma concentration than individuals carrying none or one defect *CYP2C19* allele, respectively (Yokono et al., 2001). Despite being considered pharmacologically active, the plasma concentration of the metabolite desmethylclomipramine has been reported to be inversely correlated to the clinical effect (Noguchi et al., 1993), thus raising the possibility that CYP2C19 status could influence therapeutic outcome. The SSRI sertraline is demethylated to an almost inactive metabolite, and sertraline and desmethylsertraline AUC have been shown to be 41% higher and 35% lower, respectively, in PMs compared with EMs when sertraline is given as a single oral dose (Wang et al., 2001). In vitro it appears, however, that several P450s are involved in the demethylation of sertraline, although CYP2C19 is representing the most important enzyme (Kobayashi et al., 1999; Xu et al., 1999; Obach et al., 2005). In

addition, the dose–response in sertraline treatment is poor as evident from a lack of improved therapeutic effect when increasing the dose in nonresponders (Schweizer et al., 2001), suggesting that *CYP2C19* genotype would not aid in genotype-adjusting drug dosage using sertraline. The SSRI citalopram is metabolized by CYP2C19 into a demethylated metabolite with lower plasma concentration and potency than citalopram (Hyttel, 1982). CYP2C19 preferentially metabolizes the *S*-form of citalopram that is considered to mediate the antidepressant effect, and the AUC of citalopram and *S*-citalopram (escitalopram) has been shown to be significantly higher in PMs as compared with EMs (Herrlin et al., 2003; Yu et al., 2003c). A gene–dose effect has also been shown for both citalopram and escitalopram with a difference in concentration/dose ratio, MR (drug/metabolite) and serum concentration between individuals carrying one or no defective *CYP2C19* allele (Rudberg et al., 2006). Even though a escitalopram plasma concentration–response relationship is not clear, an increased dose from 10 to 20 mg appears to increase the response rates in severely depressed compared with moderately depressed patients (Bech et al., 2006). In Table 3, a summary of the effect of CYP2C19 on antidepressants being discussed in this review is presented.

Part of the lack of correlation between antidepressant dose and/or plasma concentration with clinical effect could be attributable to mechanistically different depression phenotypes, whereby one drug type can be successfully replaced by another, as has been shown for moclobemide (MAOI) nonresponders with sertraline (SSRI) replacement therapy (George et al., 1999). This indicates that P450 genotype-adjusted drug dosage is one route by which drug treatment in depression can be improved (cf. Kootstra-Ros et al., 2006) but that other means of improving the therapeutic outcome are similarly important.

3.3. Schizophrenia

Approximately 50% of psychiatric (52%) and psychogeriatric (49%) patients use at least one drug metabolized by CYP2D6 (Mulder et al., 2007). Neuroleptics (in particular fluphenazine) and haloperidol plasma levels are influenced by CYP2D6, which is of great importance since these drugs have narrow therapeutic windows (Dorado et al., 2007). The side effects registered in relation to CYP2D6 polymorphism and treatment with the CYP2D6 substrates perphenazime, haloperidol and thioridazine are oversedation and Parkinsonian side effects, whereas no significant relationship is seen in relation to the occurrence of tardive dyskinesia, acute dystonia, extrapysimidal symptoms or akathisia (Dahl, 2002). Commonly used neuroleptics are also clozapine and olanazapine, which both have a narrow therapeutic index and are mainly metabolized by CYP1A2 (Murray, 2006). This enzyme does however not show a clear functional genetic polymorphism (see above).

Table 3
A summary on the relationships between drug concentrations, CYP2C19 metabolism, response and side effects for antidepressant drugs discussed in this review

	CYP2C19 and concentration	Concentration and response	Concentration and side effects
Moclobemide	Yes	No	Yes
Amitriptyline	Yes	No	Yes
Clomipramine	Yes	–	Yes
Sertraline	Yes	No	–
Citalopram	Yes	?	–

3.4. P450s and personality trait

An important question concerns whether the genetic polymorphism of the most important CYP enzymes for drug metabolism like CYP2C19 and CYP2D6 would affect the metabolism of endogenous compounds and thus contribute to interindividual variation in constitutive phenotype or behavior. Because of the metabolism by these enzymes of many compounds that are psychoactive, one might consider the fact that the enzymes also are active in the metabolism of endogenous substances of importance for physiological functions. Indeed, recently Ishii et al. (2007) found that *CYP2C19* genotype associates with particular phenotype traits in female Japanese subjects, but not in males. By assessing personality traits using the Temperament and Character Inventory (TCI), 3 of 7 traits were scored differently in CYP2C19 PMs and EMs. Specifically, the scores of reward dependence (RD; temperament trait), self-transcendence and cooperativeness (ST and C; character traits) were lower in PM individuals (Ishii et al., 2007), thus implying that PM subjects are more practical, tough-minded and socially insensitive. The study by Ishii et al. (2007) could be considered somewhat contradictory to another study by Yasui-Furukori et al. (2007) which did not separate the sexes and who found no association of genetically determined CYP2C19 phenotypes with the RD, ST and C traits. Instead, they found that harm avoidance (HA; temperament trait) was statistically different between EMs and PMs, with EMs having a lower score than PMs. This would imply that PMs would be more towards the classification of fearful, socially inhibited and pessimistic than EMs and seemingly opposing to the characteristics found by Ishii et al. (2007). Whether CYP2C19 is affecting personality by local metabolism in the brain or by metabolism of systemically circulating endogenous compounds is at present not known, but expression of CYP2C19 in brain remains to be specifically investigated and potential CYP2C19 substrates that may be involved in the manifestation of personality traits by CYP2C19 need to be identified. Thus, further studies are needed, both to dissect the nature of the effect of CYP2C19 on personality traits and to investigate the molecular basis for such potential effects.

The *CYP2D6* genotype has also been implicated in personality trait. Bertilsson et al. (1989) and Llerena et al. (1993) found that PMs were more anxiety-prone and less successfully socialized than EM of debrisoquine, whereas Roberts et al. (2004) and Kirchheiner et al. (2006) found that PMs are less prone to avoid harm and less careful than EMs. The difference in personality trait between CYP2D6 phenotypes appears to be more pronounced in females than in males (Kirchheiner et al., 2006), as was also shown for CYP2C19 (Ishii et al., 2007). Since CYP2D6 is expressed in brain and

involved in the metabolism of neurotransmitter substances, although at a very low affinity (Yu et al., 2003a, 2003b), a link between CYP2D6 expression level, brain transmitter levels and personality might possibly be evident, although the true nature of the association remains to be identified.

3.5. Pain

The most obvious link between the CYP polymorphism and pain treatment is the influence of CYP2D6 on the effect of codeine and tramadol treatment. Codeine has a 200 times lower affinity at μ-opioid receptors than morphine and therefore its clinical effects largely depend upon its *O*-demethylation to morphine, although some of its clinical effects appear to persist independently of morphine formation (Lotsch et al., 2006). Sindrup et al. (1993) have shown the importance of CYP2D6 enzyme activity for the pain relieving effect of codeine treatment, and CYP2D6 UMs metabolize codeine to morphine at a very high rate that can cause adverse drug reactions (Dalen et al., 1997). Tramadol is potentially a very useful pain relief medication in neonates and infants. It is primarily metabolized into *O*-demethyl tramadol by CYP2D6. The analgesic potency of tramadol is about 10% of that of morphine following parenteral administration. The *CYP2D6* genotype has been shown to determine the concentrations of *O*-desmethyltramadol enantiomers and influence efficacy of tramadol treatment (Pedersen et al., 2006; Garcia-Quetglas et al., 2007; Stamer et al., 2007). PMs for CYP2D6 showed a lower response rate to postoperative tramadol analgesia than EMs (Stamer et al., 2003). A significant effect on the tramadol efficacy is also seen in Chinese due to the partially defect *CYP2D6*10* allele (Wang et al., 2006a). Evaluation of pain threshold to single electrical sural nerve stimulation, pain summation threshold to repetitive electrical sural nerve stimulation (temporal summation), and the cold pressor test revealed that in EMs, tramadol reduced discomfort experienced during the cold pressor test, whereas in PMs, the pain tolerance thresholds to sural nerve stimulation were increased (Enggaard et al., 2006). In conclusion, CYP2D6 influences significantly the effect of the analgesic drugs codeine and tramadol and it is doubtful whether this should be a first choice treatment for pain in PMs for this enzyme.

3.6. Organ transplantation

With the use of powerful immunosuppressive drugs, organ transplantation has become the treatment of choice for many end-stage chronic organ failures. Despite the narrow therapeutic index and many side effects, the calcineurin inhibitors cyclosporine and tacrolimus are important drugs for immunosuppressive regimens. The transplantation success depends on a delicate balance between immunosuppression and rejection, and thus the maintenance of adequate blood levels of cyclosporine and tacrolimus is critical. Especially since the pharmacokinetics of these drugs is highly variable and unpredictable. CYP3A4 and CYP3A5 are involved in the metabolism of both cyclosporine and tacrolimus, and therefore, genetic polymorphisms in these genes could be associated to the elimination rate of the drugs. With respect to tacrolimus, it has been shown in several studies that the tacrolimus dose-adjusted levels showed a statistically significant difference between patients homozygous for *CYP3A5*3* compared with those carrying *CYP3A5*1* alleles, with the level in the former being higher than the latter (Macphee et al., 2002; Hesselink et al., 2003; Goto et al., 2004; Haufroid et al., 2004; Tsuchiya et al., 2004; Macphee et al., 2005; Mourad et al., 2005; Tada et al., 2005; Zhao et al., 2005). With respect to cyclosporine, one study showed that the dose-adjusted trough concentration for cyclosporine was 1.6-fold higher in *CYP3A5*3/*3* patients than in *CYP3A5*1/*3* patients (Haufroid et al., 2004). As a comparison, the dose-adjusted trough concentrations for tacrolimus were 3-fold higher in *CYP3A5*3/*3* patients in the same study. Also *CYP3A4* genotype has been implied to affect cyclosporine pharmacokinetics since patients carrying the *CYP3A4*1B* allele has been shown to have significantly higher oral cyclosporine clearance compared with patients homozygous for *CYP3A4*1A*, although this difference was small (Min & Ellingrod, 2003; Hesselink et al., 2004) and could not be reproduced in several other studies. Therefore, in contrast to tacrolimus, *CYP3A4* or *CYP3A5* genotyping is unlikely to assist in planning initial cyclosporine dosing of transplant recipients.

3.7. Rheumatoid arthritis

The drugs commonly used for the treatment of rheumatoid arthritis include NSAID, corticosteroids, slow-acting drugs and, since it is an autoimmune disease, immunosuppressive drugs. With respect to the NSAID, many of them are metabolized by the polymorphic CYP2C9, and the low activity conferred by *CYP2C9*2* and *CYP2C9*3* has been shown to influence the pharmacokinetics of ibuprofen, naproxen, diclofenac and celecoxib (Kirchheiner & Brockmoller, 2005).

3.8. GI disorders

CYP2C19 is the main enzyme involved in the metabolism of PPI, such as omeprazole and lansoprazole, and the pharmacokinetics and acid inhibitory effect of PPIs are well correlated with *CYP2C19* genotype in vivo (Furuta et al., 1999; Shirai et al., 2001; Schwab et al., 2004). As a consequence, *CYP2C19* genotype is a strong determinant for the success rate in the treatment of acid-related disorders, such as gastroesophageal reflux disease using PPI treatment only, and *Helicobacter pylori*-positive gastric ulcers using today's triple or quadruple regimens of 1 PPI and 2 or 3 antibacterial agents (Furuta et al., 2002; Kawamura et al., 2003; Take et al., 2003; Schwab et al., 2004; Furuta et al., 2005). The effect of CYP2C19 has repeatedly been proven to be manifested in lower PPI plasma levels and decreased healing rates in subjects carrying none as compared with 1 or 2 defective *CYP2C19* alleles. One study has investigated the relationship of the treatment success rate of *H. pylori*-positive peptic ulcers and the rapid *CYP2C19*17* genotype using triple therapy with pantoprazole and found no association (Kurzawski et al., 2006). By use of a correlation of omeprazole MR with AUC ($n=24$) and a larger set of

omeprazole MR data in healthy Swedish subjects ($n=107$, *CYP2C19*2* carriers and PMs excluded), it has been predicted that individuals homozygous for *CYP2C19*17* will have an almost 40% lower omeprazole AUC as compared with those homozygous for *CYP2C19*1* after a single oral dose of 20 mg (Sim et al., 2006). Since omeprazole has higher affinity to CYP2C19 than pantoprazole (Li et al., 2004), which was used in the so far only single study investigating the effect of the *CYP2C19*17* allele on PPI treatment, future studies are important in order to investigate the effect of the *CYP2C19*17* allele on ulcer treatment using omeprazole as PPI.

In Japan, where as many as 20% of the population are PMs, a prospective study of genotype-based ($n=149$) versus standard ($n=144$) therapy for *H. pylori* eradication was recently published by Furuta et al. (2007). Patients assigned to the standard protocol were given 30 mg of lansoprazole twice daily together with clarithromycin (CLA, 400 mg) and amoxicillin (750 mg). The genotype-based therapy took both the *CYP2C19* genotype and the genetically based resistance of *H. pylori* to clarithromycin into account. The duration of the standard and CLA-sensitive treatment regimens were 1 week, whereas the CLA-resistant regimen was 2 weeks. In conclusion, it was found that genotype-based therapy lead to an eradication rate of 96%, whereas it was only 70% in those treated by the standard protocol. The cost per successful eradication was similar for both strategies, indicating that a higher frequency of eradication can be achieved without any extra cost. Since there is a more than 4 times higher frequency of PMs in the Japanese population as compared with Caucasians, it is possible that a *CYP2C19* genotype test to identify carriers of defective alleles would be clinically relevant for the treatment of *H. pylori*-infected acid-related ulcers in Asia but probably not in the Western world. Since increased PPI plasma levels in PM individuals do not lead to any significant side effects, it is possible that a general increase of the standard drug dose for all Caucasian individuals would be a better approach from an economical point of view. Few studies have prospectively examined the cost–benefit of pharmacogenetic tests. There is however plenty of association studies that show the impact of genotype on drug levels. Prospective studies such as that carried out by Furuta et al. (2007) which investigate the potential benefit of genotype-adjusted drug dosing on drug efficacy and therapy outcome are thus invaluable for the future implementation of pharmacogenetics in the clinics.

3.9. Cardiovascular disease

The large group of disorders with varying pathologies that constitute the cardiovascular diseases are treated with many different classes of drugs, such as antianginals, antihypertensives, antiarrhythmics, anticoagulants, antiaggregating agents, and lipid-lowering drugs. Many of these drugs are metabolized through the polymorphic CYP2D6 and CYP2C9 enzymes. For example, the anti-vitamin K anticoagulants acenocoumarol and warfarin, in which a reduced clearance increases adverse bleeding events, are metabolized by CYP2C9 (Kirchheiner et al., 2004b; Kirchheiner & Brockmoller, 2005). *CYP2C9*2* and *CYP2C9*3* have been associated with a higher rate of major bleeding complications during initiation of warfarin therapy and with longer hospitalization times (Higashi et al., 2002; Lindh et al., 2005). The quantitative differences in total oral clearance as a consequence of *CYP2C9*2* and *3 in relation to *CYP2C9*1* can be used as a basis for pharmacokinetically derived dose adjustments. Therefore, the observed differences in pharmacokinetics could be compensated by adjustment of the dosages or the dosing intervals of the drugs, thus improving clinical outcome. Importantly, *CYP2C9* and the C1 subunit of the vitamin K epoxide reductase (*VKORC1*) genotypes are associated with the variability in the overall pharmacodynamic responses to oral anticoagulants, indicating that a pharmacogenetic-based dose adjustment could be introduced in the clinics for such drugs. For warfarin dosing regimen this could be achieved by calculations using a multivariate model including the variables of age, height, and *VKORC1* and *CYP2C9* genotypes (Wadelius et al., 2007; Fig. 11). A comparison between the multivariate-predicted dose and actual dose reveals a very significant correlation, in particular at lower warfarin doses. The main deviation from this linearity is observed at higher doses and might be explained by induction of systems contributing to the elimination of warfarin or the presence of VCORC1 variants less sensitive to the action of the drug. In addition, CYP2C9 metabolizes several antihypertensive angiotensin II receptor antagonists, such as losartan, irbesartan, candesartan and valsartan. The *CYP2C9* genotype has been shown to influence losartan metabolism and to predict the blood pressure response to irbesartan (Hallberg et al., 2002; Yasar et al., 2002).

CYP2D6 metabolizes the antianginal agent perhexiline, which has concentration-related hepatoxicity and peripheral neuropathy. A *CYP2D6* gene–dose effect on perhexiline treatment has been observed, and therefore, *CYP2D6* genotyping before therapy may help to predict perhexiline dose requirements and reduce the risk of perhexiline toxicity (Barclay et al., 2003). CYP2D6 is also involved in the metabolism of other calcium channel blockers: cinnarizine and flumarizine (Narimatsu et al., 1993). The *CYP2D6* genotype has also been shown to influence the disposition of the β-blockers carvedilol, tomolol, metoprolol and propanolol. However, the CYP2D6-related differences in pharmacokinetics did not result in any clinical differences (Huang et al., 2003; Zineh et al., 2004; Honda et al., 2005; Nieminen et al., 2005). CYP2D6 also metabolizes the antiarrhythmics encainide, flecainide, mexiletine, propafenone, and sparteine, and is involved in the metabolism of the antihypertensive agent debrisoquine.

Defective *CYP2C19* alleles lead to reduced bioactivation of the antithrombotic prodrug clopidogrel, an effect that is very prominent already in the heterozygous state. Specifically, clopidogrel treatment for 7 days reduces platelet aggregation by approximately 35% in subjects homozygous for *CYP2C19*1*, whereas the platelet aggregation in subjects heterozygous for the defective *CYP2C19*2* allele is not reduced by clopidogrel treatment (Hulot et al., 2006). Thus, subjects carrying 1 or 2 defective *CYP2C19* alleles, which amount to about 30% of Black and Caucasian populations and about 60% of Chinese subjects (Xie

Variables	Dose p
VKORC1	<.0001
CYP2C9*2,*3	<.0001
PROC	0.0461
Bodyweight	<.0001
Age	0.0002
Interactions	0.0077
Indication	0.0412

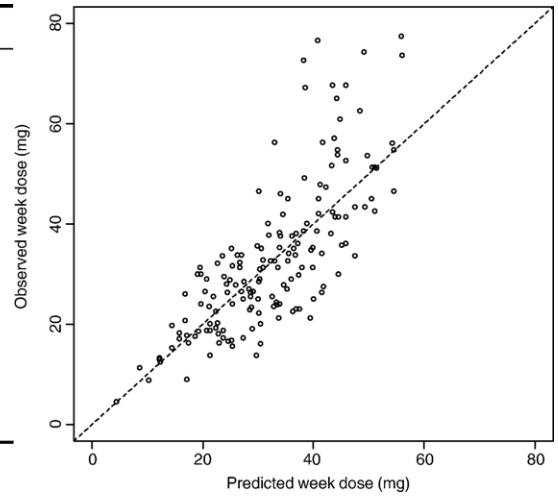


Fig. 11. Influence of polymorphism of the *CYP2C9* and *VCORC1* genes on warfarin dosage The figure shows the predicted dose of warfarin in relation to the actual dose. There is a rather good relationship, except for higher concentrations where in some cases higher warfarin doses are needed than what was predicted. An investigation of 20 000 SNPs revealed that a significant genetic association was only obtained for the genes *CYP2C9* and *VCORC1*. In total, the prediction of the warfarin dose according to the multiregression analysis is 62%. The figure was kindly obtained from Dr. Mia Wadelius, and the data have essentially been published (Wadelius et al., 2007).

et al., 2001), are not expected to respond to clopidogrel treatment at all and are thus suffering from an increased risk of recurrent thromboses.

3.10. Antiretrovirals

Frequently, an effective initial therapy for HIV infection is efavirenz, a nonnucleoside reverse transcriptase inhibitor, in addition to the administration of 2 nucleoside reverse transcriptase inhibitors. However, a high number of patients receiving efavirenz experience central nervous system side effects which may reflect varying efavirenz plasma concentrations. Efavirenz is metabolized primarily by hepatic CYP2B6 (Ward et al., 2003) and the median efavirenz AUC was in HIV-infected subjects found to be significantly different for *CYP2B6* 516G>T (Q172H) genotypes, of which the 516T mutation is more common in Africans than in Europeans (Haas et al., 2004, 2005). In addition, this polymorphism has also been associated with central nervous system symptoms (Haas et al., 2004). Consequently, plasma efavirenz levels were predicted to exceed 47 ng/mL for 121 days in 5% of subjects with the *CYP2B6* 516GG genotype, 5% of subjects with the GT genotype, and 29% of subjects with the TT genotype (Ribaudo et al., 2006). Thus, this indicates that the *CYP2B6* 516T genotype is associated with a significantly greater efavirenz plasma exposure during HIV therapy, which may lead to increased susceptibility to efavirenz-mediated side effects in the central nervous system side effects and an increased risk of developing drug resistance in patients who discontinue efavirenz-containing regimens. In Africans, the *CYP2B6*16* allele is rather common (16%) and the resulting enzyme is less efficiently expressed than the CYP2B6.1 enzyme (Wang et al., 2006b). Thus, individuals carrying this allele, in particular in combination with the *CYP2D6*6* allele, obtain much higher plasma efavirenz levels at standard dosing as compared with subjects carrying other *CYP2B6* genotypes (Wang et al., 2006b). A clinical application of this knowledge might make anti-HIV treatment more effective.

Nelfinavir, another common drug used for treatment of HIV, is metabolized mainly by CYP2C19, and plasma exposure to nelfinavir has been associated with the defective *CYP2C19*2* allele (Haas et al., 2005).

3.11. Other examples

CYP2C9 is the main enzyme catalyzing the biotransformation of many oral antidiabetics, such as sulphonylureas and nateglinide. The total oral clearance of the sulphonylureas tolbutamide, glyburide, glimepiride and glipizide has in persons with the *CYP2C9*3/*3* genotype been shown to be about 20% of that of those with the wild type genotype *CYP2C9*1/*1*, whereas the clearance in heterozygous carriers was between 50% and 80% of that of the wild-type genotype (Kirchheiner et al., 2005). Therefore, adverse effects of many oral antidiabetics may be reduced by *CYP2C9* genotype-based dose adjustments.

The benzodiazepines are a class of drugs with sedative, anxiolytic and anticonvulsant properties. Diazepam is used to treat preoperative anxiety, and PMs of CYP2C19 have been shown to take almost double the time to emerge from general anesthesia than EM individuals, due to a 35% lower clearance of diazepam and thus prolonged sedation in these patients (Inomata et al., 2005). Etizolam is a related compound being used for anxiety disorders and the pharmacokinetics of single doses are affected by *CYP2C19* genotype. Thus, PMs demonstrate a higher etizolam AUC than EMs and a higher level of etizolam-induced sleepiness as determined by test scores (Fukasawa et al., 2005). Clobazam is used in the treatment of epilepsy and is mainly converted to *N*-desmethylclobazam that has a longer half-life than the parent drug and is considered to possess partial pharmacodynamic effects, in addition

to exerting much of the side effect characteristics of the drug (Bun et al., 1986). CYP2C19 catalyzes the further metabolism of *N*-desmethylclobazam to 4-hydroxydesmethylclobazam, a route that is drastically impaired in PM subjects, thereby potentially causing an increased risk of side effects such as drowsiness (Contin et al., 2002; Kosaki et al., 2004).

4. Implementations

4.1. Methods for cytochrome P450-single-nucleotide polymorphism detection

Genotyping *CYP* genes can be difficult, due to the high sequence similarity that exist between the different *CYP* genes within the same subfamily. Therefore, careful controls of the primary polymerase chain reaction (PCR) products are necessary to guarantee that the result obtained correspond to the right target. Special attention should be given to *CYP1A1* and *CYP1A2*; *CYP2A6* with *CYP2A13* and the pseudogene *CYP2A7*; *CYP2B6* with the pseudogene *CYP2B7;* the 4 *CYP2C* genes (*CYP2C8, CYP2C9, CYP2C18* and *CYP2C19*); *CYP2D6* due to the pseudogenes *CYP2D7* and *CYP2D8;* and the 4 *CYP3A* genes (*CYP3A4, CYP3A5, CYP3A7, CYP3A43)* and the pseudogenes *CYP3AP1*, *CYP3AP2* and *CYP3AP3*.

With respect to the different techniques that can be used for SNP detection, a large number of different technologies have been developed over the past years, based on various methods of allelic discrimination and detection platforms. The application of each technique is largely dependent on the number of SNPs to be screened and on the sample size. Commonly used gel electrophoresis-based genotyping methods for known polymorphisms include PCR followed by restriction fragment-length polymorphism analysis, allele-specific amplification, and oligonucleotide ligation assay. Because of the ease of experimental assay design, SNP genotyping methods based on single-base extension are in rapid development, such as fluorescence homogenous assays, pyrosequencing and mass spectrometry. Genotyping assays such as the Invader trade mark assays allow for genotyping directly from genomic DNA without the requirement of PCR amplification. With respect to the detection of CNV (e.g., *CYP2D6* deletions, duplications), there are currently newer techniques in addition to the initial Southern blot and long-range PCRs, the main examples of which are quantitative PCR and pyrosequencing, wherein the intensity of the target-gene signals are compared with that of a reference gene (Schaeffeler et al., 2003; Soderback et al., 2005). Today, the main companies in the field provide with real-time PCR-based assay methods for most clinically important variant genes of CYP2C9, CYP2C19 and CYP2D6.

The challenges for genotyping in the near future include increasing speed of assay development, while requiring less sample manipulation, reducing the cost of the assays, and performing multiple assays in parallel (multiplexing), thus reducing the assay cost. These will fundamentally change the practice of medicine by allowing physicians to prescribe medicine based on a patient's easily achieved genetic test.

4.2. Clinical use of cytochrome P450 genotyping

The question that is most often asked is how the knowledge of CYP genotyping is translated into a clinical setting. In a recent review, Sjoqvist and Eliasson (2007) summarize their experience from genotype analyses at Huddinge University Hospital. Studying predictive and retrospective genotyping of 842 patients with primarily psychiatric disorders treated with neuroleptics and antidepressants that are CYP2D6 substrates, they identified 60 CYP2D6 PM cases where the genotype analyses was sufficient in 57% to fully explain or to contribute to the understanding of the therapeutic problem or failure in question. They found 19 UMs among 326 patients tested (5.5% instead of 1.5% expected), of which 17 UMs identified could fully explain or contribute to the therapeutic problem. This implies that such genotyping analyses, provided that the cost would continue to decrease, definitely has a large potential to contribute with a very valuable aspect in making a more efficient and safer drug therapy in the psychiatric clinic possible.

4.3. Food and Drug Administration/European Agency for Evaluation of Medicinal Products guidelines

A critical point in CYP pharmacogenetics is how the knowledge should be translated into clinical practice and into drug development. With respect to the implementation of the latter a critical role is of course assigned to the regulatory agencies, e.g., Food and Drug Administration (FDA) and European Agency for Evaluation of Medicinal Products (EMEA). FDA has developed guidelines for industry concerning pharmacogenomic data submission that can be found at www.fda.gov/cber/gdlns/pharmdtasub.pdf. The document provides recommendations for industry holding investigational new drug applications (IND), new drug applications (NDA), and biologics license applications (BLA) on (1) when to submit pharmacogenomic data to FDA during the drug development and review processes, (2) what format and content to provide for submissions, and (3) how and when the data will be used in regulatory decision making. This submission is hitherto voluntarily based; however, the aim is to integrate pharmacogenomics into drug development. Thus, if a pharmacogenomic test shows promise for enhancing the dose selection, safety, or effectiveness of a drug, FDA support that the industry fully integrates pharmacogenomic data into the drug development program. FDA states that this might include the following:

(1) Inclusion of the pharmacogenomic data in the drug labeling in an informative manner.
(2) Use of the pharmacogenomic data to choose a dose and dose schedule to identify patients at risk or to identify patient responders. This would require codevelopment of the drug and the pharmacogenomic test to be used. The schedule could include:

- testing of patients for drug metabolism genotype and dosing according to the test results;

- selection of patients as potential responders for an efficacy trial based on genotype or gene expression profile;
- exclusion of patients from a clinical trial based on genotype or gene expression profile (e.g., biomarker for addressing the risk of an adverse event).

FDA makes pharmacogenomic labels of drugs where the pharmacogenomic data would be of high importance for drug treatment. The current labels include those for the anticancer drugs herceptin (trastuzumab), irinotecan and 6-mercaptopurines, but this variability does however not involve CYP polymorphism. FDA has a label for strattera (atomoxetine) that is used for treatment of Attention Deficit Hyperactivity Disorder (ADHD) where the dosage is highly dependent on the CYP2D6 geno/phenotype. In addition, a label for treatment with warfarin (CYP2C9 and VKORC1) and tamoxifen (CYP2D6) are discussed. FDA concludes that among the metabolizing enzymes, TPMT and CYP2D6 are valid biomarkers but with the exception for Strattera no definite CYP pharmacogenetics label has been decided.

The Committee for Medicinal Products for Human use (CHMP) at EMEA released a reflection paper has been released by the committee for medicinal products for human use (CHMP) concerning the use of pharmacogenetics in the pharmacokinetic evaluation of medicinal products (www.emea.eu.int/pdfs/human/pharmacogenetics/12851706en.pdf). This paper is just finalized (July 2007) and will probably be transferred into guidelines during 2008. It emphasizes that if relevant pharmacogenomic-related differences are present for a medicinal product, the presentation of the pharmacogenomic/kinetic results should include a clear description of the alleles studied from biological and genetic perspectives, and a presentation of the pharmacokinetics or clinical parameters studied using appropriate statistics. EMEA claims that the assessment of clinical consequences of any observed difference in drug exposure in a subpopulation should be based on, e.g., the magnitude of the difference in exposure, the relationship between drug exposure and clinical effects/adverse effects, the severity of the possible adverse events and clinical consequences of loss of efficacy. The assessment could be based on clinical dose-ranging studies, on pharmacokinetic/dynamic studies, and on clinical data obtained in the genetic subpopulation and the study population as a whole. In general, the pharmacogenomic basis for differences in drug pharmacokinetics or drug effects should be provided in the Summary of Product Characteristics (SPC) if possible. If variability is at hand but with unknown genetic basis, samples should be collected for further future analysis of the pharmacogenomic background.

4.4. Indications for use of pharmacogenetics in the clinics

In the implementation procedure of pharmacogenetics into drug development and drug usage, one has to separate the issues regarding old and new drugs. With respect to the new drugs, it is clear that relevant pharmacogenetics issues will be well taken cared of during development and during release into the market by the action of the regulatory agencies. Firstly, fewer drugs will be developed where the pharmacogenetic aspect is crucial for dosage or effects since such candidates are likely to be eliminated early in drug development. Initial screening for polymorphic enzymes is already done early in development by most pharmaceutical companies. Secondly, if it is indeed impossible to avoid the interactions with a polymorphic enzyme, the drug will be released on the market together with a pharmacogenomic test to be used. This ensures that the prescription needed for outliers would be appropriate from a genetic standpoint.

With respect to the old drugs, the situation is more complicated. Here, the regulatory agencies cannot take a similar direct lead. Pharmacogenetic tests can be recommended but the major success of the implementation is usually at the hand of the physicians. These would be more prone to use recommendations for pharmacogenetic-based dosing if there were large prospective studies that showed a clear benefit of genetic dosing for better efficacy. But because of the large costs for such studies and the relative lack of interest from industry, only a few prospective large pharmacogenetic studies are currently at hand. On the other hand, the data arising regarding the survival rates among breast cancer patients taking tamoxifen where PMs for CYP2D6 have a much lower expectance for survival due to the inability to convert the drug into its active metabolite endoxifen (Borges et al., 2006; Goetz et al., 2007), it is our opinion that in such cases the pharmacogenomic knowledge has to be implemented in the routine cancer treatment regimens rapidly.

Table 4
A summary of the impact of genetic polymorphism of the different hepatic CYP enzymes

Enzyme	Estimated known genetic fraction to enzyme variability in vivo	Most important allelic variants	Estimated genetic fraction of the allele to the genetic component of enzyme variability	Comments
CYP1A2	0–10%	*CYP1A2*1F*	5%	Influences Inducibility
		*CYP1A2*1K*	10–15%	Reduced activity allele
CYP2B6	10–30%	*CYP2B6*6*	5–10%	Data not conclusive
		*CYP2B6*16*	10–30%	Only in Africans
CYP2C9	50–70%	*CYP2C9*2*	40–50%	Reduced activity allele
		*CYP2C9*3*	70–90%	Major defect CYP2C9 allele
CYP2C19	50–70%	*CYP2C19*2*	100%	Defect allele
		*CYP2C19*3*	100%	Defect allele
CYP2D6	see Table 2	Many	0–100%	20 important allelic variants
CYP3A4	5–10%	*CYP3A4*1B*	10–20%	Influences gene expression
CYP3A5	1–25%	*CYP3A5*3*	80–90%	Aberrant splicing

5. Conclusions

The genetic variability of the *CYP2C9*, *CYP2C19* and *CYP2D6* genes can be estimated to significantly influence about 20–25% of drug treatment to such a large extent that they are of clinical importance for the outcome of drug therapy. The polymorphism of the different CYPs translates into interindividual variability to different extents depending on the enzyme in question and the impact of the allelic variant (see Table 4). Among the particularly important treatment regimens affected by these polymorphisms are therapies with several antidepressants, antipsychotics, antiulcer drugs, anti-HIV drugs, anticoagulants, antidiabetics and the anticancer drug tamoxifen. The pharmacogenetic influence is to a large extent dependent on the specific drug in question, and it would be relevant to work out specific protocols for pharmacogenetically labeled drugs where the relevance for genetic analyses for the dosing and choice of drug treatment should be given. We think that it is likely that we today know of the majority of genetic factors of importance for interindividual variation in enzyme activity of the gene products encoded by the *CYP2A6, CYP2B6, CYP2C9, CYP2C19* and *CYP2D6* genes, whereas unknown genetic and/or epigenetic mechanisms have to be unraveled before we can get an understanding about the variability in CYP1A2 and CYP3A4 catalyzed reactions. Our knowledge about CYP polymorphism is however already now sufficient to constitute a very useful instrument for a more efficient drug therapy which would benefit millions of patients worldwide.

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