

Cancer treatment and pharmacogenetics of cytochrome P450 enzymes

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Summary

For the treatment of cancer, the window between drug toxicity and suboptimal therapy is often narrow. Interindividual variation in drug metabolism therefore complicates therapy. Genetic polymorphisms in phase I and phase II enzymes may explain part of the observed interindividual variation in pharmacokinetics and pharmacodynamics of anticancer drugs. The cytochrome P450 superfamily is involved in many drug metabolizing reactions. Information on variant alleles for the different isoenzymes of this family, encoding proteins with decreased enzymatic activity, is rapidly growing. The ultimate goal of ongoing research on these enzymes would be to enable pharmacogenetic screening prior to anticancer therapy. At this moment, potential clinically relevant application of CYP450 pharmacogenetics for anticancer therapy may be found for *CYP1A2* and flutamide, *CYP2A6* and tegafur, *CYP2B6* and cyclophosphamide, *CYP2C8* and paclitaxel, *CYP2D6* and tamoxifen, and *CYP3A5*. For this latter enzyme, the drugs of interest still need to be identified.

Introduction

Drug therapy is usually based on the assumption that each individual will metabolize drugs with the same efficiency, although this is often not the case. Especially when the therapeutic window is narrow, and the consequences of over- or underdosing are severe, additional information predicting metabolism may optimize individual drug therapy. Factors like diet, co-medication, health status, renal and liver function, and genetic background may affect the outcome of therapy. The contribution of each of these components may differ between drugs, and between individuals. However, information on genetic background can be obtained relatively easy nowadays, and may thus help in optimizing drug therapy. Many drugs are substrates for phase I (oxidative) and/or phase II (conjugative) metabolizing enzymes. Variant alleles in the population coding for these enzymes cause individuals to differ considerably in metabolic capacity. Variant alleles of the thiopurine methyltransferase (*TPMT*) gene are well known for their consequences on 6-mercaptopurine and azathioprine therapy [1], variant alleles for dihydropyrimidine dehydrogenase (*DPYD*) may predispose to toxic side effects for 5-fluorouracil therapy [2] and the UDP-glucuronosyl transferase (*UGT*) *1A1* genetic polymorphisms interfere

with irinotecan metabolism [3]. For the phase I enzymes, the cytochrome P450 (CYP450) superfamily is an important group of enzymes with respect to drug metabolism. It consists of over 60 structurally related enzymes, which are grouped in subfamilies. These enzymes catalyse the oxidation of many exogenous and endogenous compounds, and are responsible for the metabolism of over 90% of clinically prescribed drugs. Knowledge on CYP450 variant alleles in the population is rapidly increasing in the last few years, making them interesting as targets for genetic screening. In a substantial amount of patients, variant alleles for CYP450 enzymes may affect the pharmacokinetics of prescribed drugs. For instance, 5–10% of the Caucasian population is deficient in *CYP2D6* activity (poor metabolizers; PMs) due to having two inactive alleles. Considering that 20% of all commonly prescribed drugs are metabolized by this enzyme, it illustrates the potential contribution of genotyping to optimize individual drug therapy. It is believed that a substantial reduction of the 100,000 adverse drug reactions seen in the USA each year is possible by upfront knowledge of the drug metabolizing capacity of specific individuals. For such a pharmacogenetic screening to be effective, however, it is important that the CYP450 enzyme contributes significantly to the metabolism of the specific drug,

that there is a genetic polymorphism known that affects the metabolic activity of the enzyme to a major extent, and that such a polymorphism occurs frequently enough (relative to the clinical consequence of impaired metabolism) to warrant screening. This paper describes the current knowledge about genetic polymorphisms in the main cytochrome P450 enzymes, and their relation to anticancer therapy.

CYP1A2

Cytochrome P450 1A2 (CYP1A2; chromosome 15q22-pter) accounts for approximately 15% of the total CYP450 protein content in human liver [4], and is involved in the metabolism of more than 20 clinically used drugs, including caffeine, theophylline, clozapine and propranolol. Large interindividual variation in expression has been observed (40-fold based on mRNA [5]) and CYP1A2 displays higher activity in men compared to women. At present, 16 variant alleles have been documented, in which the **1C*, **1K*, **7* and **1I* alleles encode decreased activity enzymes while the **1F* allele encodes a high inducibility allele (www.imm.ki.se/CYPalleles; (Aug. 1, 2005)). The antiandrogenic drug flutamide, which is widely used in the treatment of prostate cancer, is activated by hydroxylation by CYP1A2 [6]. Individuals with decreased CYP1A2 activity due to inheritance of decreased activity alleles could theoretically benefit less from flutamide therapy, in which presumably the inheritance of at least two decreased activity alleles would be necessary to get a clinically significant reduction of metabolism. The **1C* variant has an allele frequency of 23% in the Japanese population [7], the **1K* variant has an allele frequency of 3% in Ethiopians, 3.6% in Saudi Arabians and 0.5% in Spaniards [8], the **7* allele apparently has an allele frequency below 0.5% [9] and the **1I* allele has an allele frequency of 0.2% in the Japanese population [10]. Further studies are needed to clarify the effect of these SNPs is on efficacy of flutamide therapy.

CYP2A6

Cytochrome P450 2A6 (CYP2A6; chromosome 19q13.2) plays a major role in the metabolism of nicotine and coumarin. However, also the bioactivation of tegafur, a prodrug of 5-FU, is catalyzed by CYP2A6 [11, 12]. There are 26 known allelic variants of the *CYP2A6* gene at present that may affect enzyme activity or gene expression. Among the alleles encoding absence of CYP2A6 activity are the *CYP2A6*2*, **4*, **5* and **20* alleles, while the **6*, **7*, **9*, **10*, **11*, **12*, **17*, **18A/B* and **19* alleles encode reduced activity enzymes

(www.imm.ki.se/CYPalleles) The frequencies of the variant alleles may differ between ethnicities: the **2* allele is found rather equally in Caucasians (1.1–3.0%) and in African North Americans (0.3–1.1%) and Asians (0–0.7%) [13]. The **4* allele, however, is found more often in Chinese (allele frequency 6.7–15.1%) compared to Caucasians (0.5–1.2%) or African North Americans (1.9%) [13]. It is the **1I* reduced activity allele (V_{\max} approximately half compared to wild type) that was discovered in an individual that showed a poor metabolic phenotype on tegafur therapy: this compound heterozygote *CYP2A6*4C/*1I* patient had a 4-fold higher tegafur AUC compared to other patients [14]. Based on the bioactivating role of CYP2A6, one could expect that CYP2A6 poor metabolizers would respond less well on tegafur therapy, although this hypothesis needs to be verified in future studies.

CYP2B6

CYP2B6 activity may vary considerably between individuals, and a 1.7 times higher enzyme activity was demonstrated in females compared to males [15]. Based upon (S)-mephenytoine *N*-demethylation, 7% of females ($n = 28$) and 20% of males ($n = 45$) proved to be CYP2B6 PMs [15]. For the *CYP2B6* gene, located on chromosome 19q13.2, nine SNPs were initially described in 2001, composing variant alleles **1* through **7* [16]. This has increased to 25 variant alleles at present (www.imm.ki.se/CYPalleles). For CYP2B6, results on individual single nucleotide polymorphisms (SNPs) and on complete haplotypes are reported, making comparisons between studies not always as easy. The *1459C > T* genetic polymorphism (Arg487Cys), present in **5* and **7* alleles, corresponds to lower CYP2B6 protein levels in heterozygous and homozygous variant individuals when compared to *CYP2B6*1* wild types [16]. According to Hiratsuka et al. [17], the allele frequencies in Caucasians are 11% and 3% for *CYP2B6*5* (Arg487Cys) and **7* alleles (Gln172His, Lys262Arg, Arg487Cys) respectively, while in Japanese frequencies were found of 1% (**5*) and 0% (**7*). For the *1459C > T* (Arg487Cys) polymorphism in Caucasians, other groups reported allele frequencies of 14% [16] and 13% [15]. With respect to affecting enzymatic activity, Lamba et al. [15] interestingly showed a correlation between the *1459C > T* SNP (**5* and **7* alleles) and CYP2B6 activity in Caucasian females ($p = 0.0015$), whereas this correlation was not found in Caucasian males. Either the power of the analyses in the male group was insufficient to detect a correlation between this SNP and CYP2B6 activity, or else this result may suggest that other factors are involved in the phenotypic expression of CYP2B6

variant alleles. Other authors showed that *CYP2B6**6/*6 homozygous individuals (Gln172His, Lys262Arg) have low CYP2B6 protein levels [18] (allelic frequency *CYP2B6**6 allele 26% (Caucasians) and 16% (Japanese) [17]). The encoding of decreased CYP2B6 activity by this allele is supported by the high plasma concentrations of the CYP2B6 substrate efavirenz which is found in *CYP2B6**6/*6 individuals [19] but is however not confirmed by pharmacokinetic data on bupropion, in which *CYP2B6**6/*6 individuals do not differ from *CYP2B6**1/*1 patients [20]. Efavirenz studies further indicated that presence of the 516G > T SNP (encoding Gln172His, present in *6, *7, *9 and *13 alleles) was correlated to a factor 3 decreased activity of CYP2B6 compared to *CYP2B6**1/*1 individuals. The presence of a *5 allele had no effect in this study [20]. An increase in enzymatic activity has been reported for the *CYP2B6**4 (785A > G, Lys262Arg) variant allele, as determined by bupropion hydroxylation [20]. Population kinetic analysis in that study showed that the CYP2B6 dependent hydroxylation did not differ between *CYP2B6**1, *2, *5 and *6 alleles, but that clearance by *CYP2B6**4 allele carriers (*CYP2B6**1/*4 heterozygotes) was 1.6 fold higher compared to wild type *CYP2B6**1/*1 individuals. Both the *4 and *6 variant allele carry the 785A > G mutation (encoding Lys268Arg), which may thus be responsible for the increase in enzymatic activity. Apparently, the additional Gln172His mutation in the *CYP2B6**6 allele decreases protein expression by which the net effect on CYP2B6 activity is apparently compensated in this allele. The allelic frequency these authors reported for male Caucasians was 5.0% for *CYP2B6**4, 9.5% for *CYP2B6**5, 25% for *CYP2B6**6 and 0% for *CYP2B6**7 [20]. A *CYP2B6**4 allele frequency of 5% was also reported by Lamba et al. [15], although others reported an allelic frequency in Caucasians of 32.6% [16].

CYP2B6 is involved in the activation of the anticancer drugs cyclophosphamide (CPA) and ifosfamide [18]. The difference between the sexes is also here reflected by a difference in the bioactivation of the anticancer drug ifosfamide: a 2-fold higher N-dechloroethylation activity in microsomes obtained from females compared to males [21]. Regarding the conversion of cyclophosphamide to 4OH-cyclophosphamide as a function of total CYP450 protein, however, no difference was observed between *CYP2B6**1/*1 individuals compared to *CYP2B6**6/*6 individuals [18], arguing against *CYP2B6**6 screening for cyclophosphamide treatment. For the 1459C > T SNP, present in the *CYP2B6**5 and *7 alleles, correlation with low activity *in vivo* needs to be examined while also the increased activity of the *CYP2B6**4 allele deserves more study. Allele frequencies reported differ significantly between studies, but are high enough to warrant further investigation. Apparently, for CYP2B6, differences be-

tween male and female subjects do need to be taken into consideration. The decreased activity of the *CYP2B6**8, *11, *12, *13, *14 and *15 alleles awaits further investigations with respect to effect on anticancer drug metabolism.

CYP2C8, 2C9 and 2C19

The metabolism of 20% of clinically used drugs is catalyzed by the CYP2C family. This family consists of 4 members: CYP2C8, CYP2C9, CYP2C18 and CYP2C19, for which the genes are clustered on chromosome 10q24. Polymorphisms in the *CYP2C8*, *2C9* and *2C19* genes may result in toxicity in affected individuals exposed to CYP2C substrates [22]. The first genetic polymorphism in *CYP2C19* was discovered due to abnormal metabolism of the anticonvulsant drug mephenytoin. Based on the metabolism of this drug, 3–5% of the Caucasians and 12–23% of Asians appeared to be PMs. A splice site mutation in exon 5 (*CYP2C19**2) and a premature stopcodon in exon 4 (*CYP2C19**3) represent the two most predominant null alleles [23, 24]. Up to now, at least 15 variant alleles have been described (www.imm.ki.se/CYPalleles), of which seven (*CYP2C19**2 through *8) encode no enzyme activity. With respect to anticancer therapy, CYP2C19 plays a role in the metabolism of cyclophosphamide, ifosfamide and thalidomide (Table 1) [25–27].

For CYP2C9, the principal CYP2C in human liver [28], variant alleles *2 through *20 are known. Two variant alleles (*2 (430C > T; Arg144Cys) and *3 (1075A > C; Ile359Leu) that have been shown to affect CYP2C9 metabolism *in vivo*, occur in 11% and 3–16% of whites, and in 3% and 1.3% of blacks, respectively [29]. The *CYP2C9**3 allele seems to exhibit the largest change in catalytic activity; *CYP2C9**3/*3 PMs among Caucasians occur at a frequency of approximately 0.3% [30]. The *CYP2C9**4 allele (1076T > C; Ile359Thr) and *CYP2C9**5 (Asp360Glu) allele displayed altered metabolism of diclofenac *in vitro* [31, 32]. The *CYP2C9**4 allele is a rare allele identified only in one Japanese subject [33], and has not been found in Caucasians, African Americans or Chinese [29]. The *5 allele was found with a frequency of 1.7% in African Americans and is absent or extremely rare in Caucasians [29, 32]. The nucleotide changes in the *CYP2C9**3, *4 and *5 alleles affect two amino acids close together: amino acid 359 and 360. The *CYP2C9**6 allele (818delA) encodes a frameshift, and is thus a true *CYP2C9* null allele. This allele, identified in an African American exhibiting severe drug toxicity on normal phenytoin dosages, was found in 0.6% of African Americans but could not be demonstrated in 172 Caucasians [34]. With respect to anticancer therapy, a 3-fold lower intrinsic clearance for cyclophosphamide in a yeast expression system was observed with recombi-

Table 1. CYP450 enzymes and anticancer agents. Drugs in italics indicate minor pathway

CYP450	Substrate	Ref.
CYP1A2	Flutamide (activation)	[6]
	<i>Etoposide</i>	[98]
CYP2A6	Tegafur (activation)	[11, 12]
	<i>Cyclophosphamide</i>	[99]
	<i>Ifosfamide</i>	[99]
CYP2B6	Cyclophosphamide (activation)	[99]
	<i>Ifosfamide (activation)</i>	[99]
	<i>Tamoxifen</i>	[51, 100–102]
CYP2C8	Paclitaxel/Taxol	[35, 103]
	<i>Cyclophosphamide</i>	[99]
	<i>Ifosfamide</i>	[99]
CYP2C9	<i>Cyclophosphamide</i>	[99, 104, 105]
	<i>Ifosfamide</i>	[99]
	<i>Tamoxifen</i>	[51, 100–102]
	<i>Tegafur (activation)</i>	[11, 12]
CYP2C19	Thalidomide	[27]
	<i>Cyclophosphamide</i>	[25, 105]
	<i>Ifosfamide</i>	[105]
	<i>Tamoxifen</i>	[51, 100–102]
CYP2D6	Tamoxifen (activation)	[51, 100–102]
	<i>Gefitinib/Iressa</i>	[106]
CYP2E1	<i>Etoposide</i>	[98]
CYP3A4/5	Cyclophosphamide ((in)activation)	[99, 104, 107]
	<i>Ifosfamide ((in)activation)</i>	[99, 107]
	Docetaxel	[92]
	<i>Etoposide</i>	[98, 108]
	Flutamide	[6]
	<i>Gefitinib/Iressa</i>	[106]
	<i>Imatinib/Gleevec</i>	[109]
	<i>Irinotecan</i>	[95–97]
	Paclitaxel/Taxol	[35, 103]
	<i>Teniposide</i>	[108]
	<i>Tamoxifen</i>	[51, 100–102]
	Vinca-alkaloids	[11, 110, 111]

nant CYP2C9.2 and CYP2C9.3 protein when compared to CYP2C9.1 protein [25]. However, no significant differences in cyclophosphamide metabolism in human microsomes obtained from *CYP2C9*2* or **3* individuals compared to those from *CYP2C9*1* homozygotes could be demonstrated [25]. For CYP2C8, the decreased activity allele *CYP2C8*2* (805A > T; Ile269Phe) was found predominantly in African Americans (allele frequency 18%; $n = 82$) and not in Caucasians (0%; $n = 170$). The variant *CYP2C8*3* allele (416G > A and 1196A > G; Arg139Lys and Lys399Arg), in contrast, had allele frequencies of

13% in Caucasians and 2% in African Americans. For the anticancer drug paclitaxel, decreased activity of recombinant CYP2C8.2 and CYP2C8.3 enzymes was found. For paclitaxel, 85% of the inactivation occurs by conversion to 6 α -OH paclitaxel by CYP2C8 [35]. A *CYP2C8*4* (792C > G; Ile264Met) allele with probably decreased activity has been reported [36], while a potential inactive *CYP2C8*5* allele (475delA; frameshift) was found in one Japanese individual [37]. The clinical consequences of these variant alleles still needs to be determined.

CYP2D6

Substantial interindividual variation in the metabolism of the antihypertensive drug debrisoquine, already observed in 1977 [38], led to the identification of a genetic variant allele for the responsible enzyme cytochrome P450 2D6, the *CYP2D6*4* allele [39, 40]. Presently, the *CYP2D6* gene (located on chromosome 22q13.1) is the best-studied member of the CYP450 superfamily, with over 40 variant alleles (*1 through *43). These include 26 null alleles (alleles encoding non-functional CYP2D6) and 6 alleles encoding enzymes with decreased activity (www.imm.ki.se/CYPalleles). In addition, *CYP2D6* gene exist in the population and this is correlated with ultrarapid metabolism [41]. Approximately 1–2% of the Swedish Caucasian population does have such duplication [42]. This incidence increases, going north to south in Europe, from 3.6% in Germany [43] to 7–10% in Spain [44, 45] and 10% in the south of Italy [46]. The incidence of gene duplication is even higher in Saudi Arabians (20%) [47] and black Ethiopians (29%) [48]. Interestingly, the ultrarapid phenotype in Ethiopians with *CYP2D6* duplication (metabolic ratio for debrisoquine 0.1–1) is not as extreme when compared to Caucasians (metabolic ratio 0.01–0.02) [49]. In general, 71% of *CYP2D6* alleles in Caucasians are functional alleles, while non-functional alleles represent 26%. In contrast, in Asians only ~50% of the *CYP2D6* alleles are functional [49]. The reduced function allele *CYP2D6*10* has an allelic frequency of ~40% in Asians, causing a population shift towards a lower mean CYP2D6 activity. For African-Americans and Africans, reduced function alleles represent 35% of *CYP2D6* genes, with *CYP2D6*17* being the main contributor [49]. Because alleles encoding non-functional or decreased function enzymes affect CYP2D6 metabolic potential, knowledge of CYP2D6 genetic make-up may help in optimizing therapy. Although CYP2D6 only contributes 2% of total liver CYP450 protein, this enzyme metabolizes 20% of all commonly prescribed drugs. Which genetic variant does one need to take into consideration? As indicated, interethnic differences in variant alleles (see [49] for review) complicate

a clear-cut answer. For Caucasians, the non-functional *CYP2D6**4 (allele frequency 20%) is carried by 75% of *CYP2D6* poor metabolizers (PMs). Investigating Caucasians for the presence of the non-functional alleles *3 (allele frequency 1–2%), *4 (20%), *5 (3.8%) and *6 (1%) will identify >98% of *CYP2D6* PMs. For Asians and African Americans, as mentioned above, the reduced function alleles *CYP2D6**10 and *17 should be taken into account, respectively. Finding homozygosity for non-functional *CYP2D6* alleles will predict PMs, but given the many *CYP2D6* variant alleles that exist, it may be clear that the absence of finding non-functional alleles only decreases the chance of being a PM without ever excluding it completely. Recently, a new approach for Caucasians was described, in which analysing for the (–1584)C > G polymorphism in the 5′-untranslated region seems to predict non-PMs with a positive predictive value of 1.0. When individuals are heterozygous (–1584)CG or homozygous (–1584)GG for this polymorphism, there is a 12% chance of being a *CYP2D6* PM [50].

With respect chemotherapy, *CYP2D6* does not seem to play a major role: it is involved in the metabolism of tamoxifen and gefitinib (Iressa) (Table 1). For tamoxifen treatment, *CYP2D6* activity might be of importance: tamoxifen can either be converted to the inactive N-desmethyltamoxifen by *CYP3A4* (~90% of tamoxifen metabolism) or can be activated to 4OH-tamoxifen by *CYP2D6* (~10% of tamoxifen metabolism). 4OH-Tamoxifen has a 50–100 fold higher activity as an anti-estrogen when compared to tamoxifen itself [51]. Both N-desmethyltamoxifen and 4OH-tamoxifen can be converted to endoxifen (4OH-N-desmethyltamoxifen) by *CYP2D6* and *CYP3A4*, respectively [52]. Endoxifen is thought to contribute considerably to the therapeutic effect of tamoxifen and its formation thus depends on *CYP2D6* activity [52]. Indeed, significantly lower plasma levels of endoxifen were found in *CYP2D6* poor metabolizers, compared to heterozygotes or wild types [53]. However, so far no correlation could be demonstrated between *CYP2D6* genotype and survival [54, 55].

CYP3A4, 3A5, 3A7 and 3A43

The *CYP3A* subfamily, which represents the majority of *CYP450* protein in the human liver, is responsible for the metabolism of many endogenous (testosterone, progesterone, cortisol, oestradiol) and exogenous compounds, including steroids, antidepressants, antibiotics, benzodiazepines, calcium channel blockers, HMG-CoA reductase inhibitors and anticancer drugs, in total covering over 50% of all currently used drugs [4, 56, 57]. Four human *CYP3A* genes have been identified: *CYP3A4*, *CYP3A5*, *CYP3A7* and *CYP3A43*, which cluster on chro-

mosome 7 [58]. Based on the amount of protein and catalytic potential, *CYP3A4* is the most important member of the *CYP3A* subfamily. *CYP3A4* activity shows wide inter-individual variation, up to 40-fold, which may be caused by health status, environmental (smoking, diet, co-medication), hormonal or genetic factors [4, 59]. It is thought that genetic differences may explain 60–90% of the observed variation in drug metabolizing capacity of patients [60], although up to this moment no “null” allele for *CYP3A4* has been described. Over 30 SNPs for *CYP3A4* have been published, representing alleles *1 through *19, most of which occur with allele frequencies below 5% [61]. The first genetic *CYP3A4* polymorphism described was the promoter variant allele *CYP3A4**1B (–392A > G, originally referred to as –290A > G), identified by linkage to a worse presentation of prostate cancer [62]. The allele frequency showed a large interethnic variation: 2–9% in Caucasians, 35–67% in African Americans, 0% in Taiwanese and 0% in Chinese [57, 63]. Although the *CYP3A4**1B allele was initially shown to have a 1.5-fold increase in transcription *in vitro* [64], other reports indicate no change in enzyme activity [65]. The *CYP3A4**2 allele (664T > C; Ser222Pro), described in 2000 [66], was found in 2.7% of Finnish Caucasians and was shown to encode a protein with decreased activity. Other groups, however, failed to detect this variant allele in Caucasians, including one report on 70 Finnish individuals [67, 68]. In contrast, the *2 allele was recently reported to have a 4.5% allele frequency in the Portuguese population [69]. The *CYP3A4**3 allele (1334T > C; Met445Thr), also described in 2000 and originally referred to as a rare allele [66], proved not so rare, having a frequency of 1.1% in the Dutch Caucasian population [70]. At present, 18 alleles encoding amino acid changes are known (www.imm.ki.se/CYPalleles). Decreased *CYP3A4* activity has been demonstrated for the *CYP3A4**17 allele (566T > C; Phe189Ser) when analysed for 6β-OH testosterone formation in a reconstituted system [71]. Based upon the 6β-OH cortisol/cortisol ratio, there are indications that also the *CYP3A4**4 (352A > G; Ile118Val), *5 (653C > G; Pro218Arg) and *6 (831insA; frameshift) alleles encode proteins with decreased catalytic activity [72]. Upon investigating 500 Dutch Caucasians, no *CYP3A4**4, *5, *6, or *17 alleles could be detected [61], although in a mixed group of American Caucasians, an allelic frequency of 2.1% was reported for *CYP3A4**17 [71]. The *CYP3A4**4, *5 and *6 allele frequencies among Chinese were found to be 0.5–1.5% [72]. One allele with increased *CYP3A4* activity was described: *CYP3A4**18 (878T > C; Leu293Pro) [71]. This allele was not found in Caucasians [61, 71] but proved relatively abundant in Chinese (allele frequency 10%) [71]. The impact on *in vivo* *CYP3A* activity, however, remains to be established.

The next important member of the CYP3A subfamily is CYP3A5, which is expressed in only 10–40% of Caucasians. Recently, a genetic polymorphism in intron 3 of the *CYP3A5* gene was found to be responsible for this lack of expression: 6986A > G, which was named the *CYP3A5*3* allele [73, 74]. It appeared that 80% of the Caucasian population and 30% of the African American population are homozygous for this inactive *CYP3A5* allele and are thus deficient in CYP3A5 activity [73–75]. In addition, the *CYP3A5*5* (12952T > C) and the *6 allele (14690C > A) both encode frameshifts, and thus may also represent null alleles, although with much lower allelic frequency (0.0% and 0.1%, respectively, in 500 Caucasians [73–75]). In some individuals, CYP3A5 protein expression may contribute to >50% of total CYP3A protein in the liver [73, 76]. Because of this, some authors envisage an important contribution of the CYP3A5 genetic polymorphism in predicting liver CYP3A activity, while other question this [73, 77, 78]. Because there is a large overlap in substrate specificity between CYP3A4 and CYP3A5, the contribution of each CYP3A4 and CYP3A5 to total CYP3A activity will depend on both the drug under investigation and the individual taking it. In addition, CYP3A5 is also expressed extrahepatic (prostate, kidney, adrenal, pituitary) [79–82], while CYP3A4 activity is more restricted to liver and intestine [83]. Depending on the site of action of a drug, the role of CYP3A5 may thus be larger than anticipated. Having an allele (*CYP3A5*3*) encoding absence of protein, which occurs with a high frequency in an enzyme family known to be involved in the metabolism of many drugs, it might be just a matter of time before drugs are identified which may benefit from *CYP3A5* genetic screening. One of the first examples pointing in this direction is the metabolism of the immunosuppressive drug tacrolimus, which correlates with *CYP3A5* genetic constitution [84].

CYP3A7 is generally considered to be the major fetal liver CYP3A enzyme, where it accounts for 30–50% of total cytochrome P450 [4]. In the majority of individuals, hepatic expression of CYP3A7 is significantly downregulated directly after birth, but CYP3A7 mRNA and protein have also been detected in adults [76, 83, 85]. A >700-fold difference in CYP3A7 mRNA levels has been observed between individuals [86]. On average, CYP3A7 transcripts account for only 1.5% of combined CYP3A4 and CYP3A7 mRNA, but in the 11% of human livers with >25,000 mRNA CYP3A7 transcripts/ng total RNA, the contribution of CYP3A7 mRNA was about 7% [86]. The most important genetic polymorphism consists of a switch between 60 bp from the *CYP3A4* gene and the corresponding region in the *CYP3A7* gene. This fragment contains the proximal ER6 motif, which is responsible for the pregnane X receptor (PXR) and constitutively activated receptor (CAR) transcriptional activation

of *CYP3A4* [86]. The resulting *CYP3A7*1C* allele has an allele frequency of 3% in Caucasians and 6% in African Americans and is believed in heterozygote and homozygote individuals to be responsible for CYP3A7 protein expression in liver and intestine in adults [73, 86]. The functional effect *in vivo* was recently demonstrated by the fact that carriers of this variant allele had significantly lower levels of DHEAS, which is a substrate for CYP3A7 [87]. The *CYP3A7*1B* allele, which has a –314C > T change compared to the *CYP3A7*1* allele [73], was associated with increased expression only in liver [86].

The *CYP3A43* gene was cloned only recently [58, 88, 89]. It is expressed in very low levels in adult human livers, where it accounts for only 0.1–0.2% of CYP3A4 transcripts [58, 88]. Besides liver, expression was demonstrated in liver, kidney, pancreas and prostate [89]. The amino acid sequence shows 75% similarity to CYP3A4 and CYP3A5, and 71% to CYP3A7. Because of its low expression, the contribution of CYP3A43 to CYP3A mediated metabolism is thought to be minimal.

With respect to anticancer therapy, CYP3A is involved in the metabolism of many drugs, like cyclophosphamide, ifosfamide, docetaxel, paclitaxel, etoposide, irinotecan, tamoxifen, imatinib (Gleevec) and gefinitib (Iressa) (Table 1). Docetaxel is a substrate for CYP3A4 and 3A5, and its clearance correlated with CYP3A activity, as measured by the CYP3A specific drug midazolam [90] and the erythromycin breath test [91]. Interestingly, data of Goh et al. [90] showed a trend towards a higher mean docetaxel clearance value in CYP3A5 poor metabolizers compared to that of patients with one or two active *CYP3A5* alleles, which would be opposite to expectations. However, this difference proved not statistically significant in the group of 25 patients studied [90]. Other investigations showed that CYP3A4 and CYP3A5 the major metabolizing CYP450s for docetaxel, with an estimated contribution of 64–93% to total metabolism. CYP3A4 and CYP3A5 showed comparable V_{\max} values (1.17 m^{-1} and 1.36 m^{-1} , respectively) but differed by a factor of 10 in K_m value (0.91 μM versus 9.28 μM , respectively) [92]. For paclitaxel, CYP3A4 is also involved in its metabolism, but the contribution of CYP2C activity is higher, being responsible for 85% of paclitaxel metabolism [35]. In the metabolism of etoposide, which is a substrate for CYP3A4, CYP3A5 and UGT1A1, the effect of *CYP3A5* genotype was investigated recently [93]. In blacks, *CYP3A5* genotype predicted lower etoposide clearance ($p = 0.022$), but not in whites ($p = 0.862$), suggesting that also other factors play a role. Irinotecan (CPT-11) metabolism involves a side reaction of CPT-11 to 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]-carbonyloxycamptothecin (APC), which is catalyzed by CYP3A4. Inhibition of irinotecan metabolism by ketoconazole (a CYP3A4 inhibitor) and induction by

St. John's Wort (a CYP3A4 inducer) suggest that CYP3A4 may play a role in irinotecan related toxicity [94, 95]. Also CYP3A5 was shown to be involved in irinotecan metabolism, but at a lower level than CYP3A4 [96]. Consistent with this minor role, a relation between SN-38 exposure and the *CYP3A5**3 genetic polymorphism could not be demonstrated in a study on 64 cancer patients treated with irinotecan [97].

Conclusion

In the last couple of years, information on genetic polymorphisms in CYP450 enzymes is rapidly increasing. The information now available does make it possible now to study whether pharmacogenetic analyses for cytochrome P450 enzymes may be of clinical benefit for patients which should undergo anticancer therapy. At this moment, there seems to be potential relevance for genotyping *CYP1A2* for flutamide, *CYP2A6* for tegafur, *CYP2B6* for cyclophosphamide, *CYP2C8* for paclitaxel, *CYP2D6* for tamoxifen and *CYP3A5* (based upon high frequency of variant alleles and involvement of CYP3A in the metabolism of many drugs) for drugs that still need to be identified. The outcome of these studies should make clear which analyses will be suited for clinical applications in cancer therapy in the near future.

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