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Review

Genotyping and phenotyping cytochrome P450: Perspectives for cancer treatment

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ABSTRACT

As most anticancer agents display a narrow therapeutic window, patients may be susceptible to (extreme) toxicities or a lowered therapeutic outcome if not dosed adequately. Therefore, it is important to study factors which affect the pharmacokinetics and pharmacodynamics of these drugs. Among these, the contribution of genetic variation in drug metabolizing enzymes on the metabolism of anticancer agents has gathered interest, as it may potentially explain a substantial amount of interpatient variability in pharmacokinetics and drug response. Cytochrome P450, an oxidative enzyme-system involved in the breakdown of many drugs, is currently studied for correlations between genetic polymorphisms and anticancer drug metabolism. Also, alternative ways to predict the expression of cytochrome P450 have been developed (phenotyping measures) which may have additional value in creating a lowered interpatient variability, to minimize side-effects and maximize therapeutic efficacy.

1. Introduction

Half a century ago, Watson and Crick [1], the scientists who discovered the double helical structure of DNA, wondered what would be the impact of their finding. Nowadays, we realize that this discovery stood at the beginning of a new field of science, studying DNA replication, transcription and translation. As these processes are not error-free, (point) mutations (or single nucleotide polymorphisms, SNPs) in DNA are responsible for the occurrence of genetic variation which may translate into functional consequences [2]. SNPs in exons can give rise to incorporation of a variant amino acid ("missense"-mutation), or encode a stop codon ("non-sense"-mutation), leading to a truncated protein, usually devoid of activity. Incorporation or deletion of nucleotides will result in disruption of the reading frame for translation nucleotide triplets into amino acids, a "frameshift", resulting in proteins without activity. Although paradoxical at first sight, even a "silent" mutation, where no change in amino-acid formation appears, may be of clinical interest, potentially because of the existence of "linkage disequilibrium" with other (functional) mutations, or because it affects mRNA stability. And also SNPs in intron-sequences can have an effect, because they may interfere with proper splicing of mRNA.

Clinical ("genotyping") studies have been performed to connect SNPs to alterations in pharmacokinetics and/or clinical outcome (adverse events and response), for patients treated with several agents, including anticancer drugs. For a (relatively) small number of genetic polymorphisms, the relevance for oncology practice has been determined, as reviewed

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earlier [2–7]. For instance, patients with variant alleles in genes coding for the drug metabolizing enzymes dihydropyrimidine-dehydrogenase (DPD), thiopurine-methyltransferase (TPMT), and UDP-glucuronosyltransferase 1A1 (UGT1A1) may be prone to excessive toxicity when treated with (chemo-)therapeutics metabolized by these enzymes (5-fluorouracil, 6-mercaptopurine and irinotecan, respectively) [2–11]. This is due to alterations in enzymatic activity, which is usually lowered. By taking polymorphisms into account, patients with an increased risk of (extreme) adverse events should be identified before harm is done.

In this article, the influence of genetic polymorphisms in cytochrome P450 (CYP), the largest family of enzymes involved in the metabolism of several important drugs in today's fight against cancer, is discussed.

2. Genetic variation in cytochrome P450 involved in chemotherapy

2.1. *CYP2D6*

The cytochrome P450 enzyme family consists of different members and each has specific substrates. One of the best studied members of the family is CYP2D6, located on chromosome 22q13.1, for which 5–10% of the Caucasian population is deficient ('poor metabolizer') due to inheritance of two inactive *CYP2D6* alleles [12]. Substantial inter-individual variation (IIV) in the metabolism of the anti-hypertensive drug debrisoquine, already observed in 1977 [13], led to the identification of the deficient variant *CYP2D6**4* allele [14,15]. Presently, over 40 variant alleles (*1–*43), which include 26 null alleles (non-functional CYP2D6) and 6 alleles encoding enzymes with decreased activity, have been described [16]. In addition, *CYP2D6* gene duplication has been found in individuals and correlated with an ultra-rapid metabolism [17]. Approximately 1–2% of the Swedish Caucasian population, 4% of the German [18], and 7–10% of the Spanish and Italian population have this gene duplication [19–21]. Although CYP2D6 only contributes 2% of total liver CYP protein, this enzyme metabolizes 20% of all commonly prescribed drugs. With respect to anticancer treatment, CYP2D6 does not seem to play a major role. For gefitinib, imatinib and tamoxifen treatment, however, CYP2D6 activity may be of importance (Table 1 [22–24]). Tamoxifen can either be converted to the relatively inactive metabolite *N*-desmethyl-tamoxifen by CYP3A4/5 (~90% of tamoxifen metabolism) or can be activated to 4-hydroxy-tamoxifen by CYP2D6 (~10% of tamoxifen metabolism), this latter compound having a 50–100-fold higher activity as an antiestrogen when compared to tamoxifen itself [25]. *N*-desmethyltamoxifen can be metabolized by CYP2D6 to endoxifen (4-hydroxy-*N*-desmethyltamoxifen), which is believed to contribute significantly to the therapeutic effect of tamoxifen [26]. Indeed, CYP2D6 poor metabolizers had significantly lower plasma endoxifen concentrations than heterozygous and wild-type patients [27], although no correlation between CYP2D6 genotype and survival could be demonstrated [28,29]. In contrast, a recent article described the benefit of carrying a *CYP2D6* deficient allele for tamoxifen therapy in addition to adjuvant therapy compared to adjuvant therapy alone [30]. No mechanistic explanation for this unexpected result was given by the authors, and the small group of patients was mentioned as a possible confounding factor [30].

2.2. *CYP2B6*

The interest for CYP2B6 related to anticancer therapy is because of its role in the activation of cyclophosphamide and ifosfamide (Table 1 [31–35]). Based upon (S)-mephenytoine *N*-demethylation, 7% of females ($n = 28$) and 20% of males ($n = 45$) proved to have low CYP2B6 activity [36]. For the *CYP2B6* gene, located on chromosome 19q13.2, nine SNPs were initially described in 2001, composing variant alleles *1–*7 [37]. At this moment, 25 variant alleles (*1–*25) have been described [16]. This knowledge has increased the potential benefit of screening for *CYP2B6* polymorphisms prior to anticancer therapy. However, at this moment there is some controversy to which SNPs are correlated with decreased activity. The 1459C>T genetic polymorphism (Arg487Cys), present in *5 and *7 alleles, was initially found to correspond to lower CYP2B6 protein levels in heterozygous and homozygous variants [37]. Interestingly, a correlation was found 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 [36]. Other authors showed that *CYP2B6*6/*6* homozygous individuals (516G>T, 785A>G; Gln172His, Lys262Arg) have low CYP2B6 protein levels [31]. These findings are in line with the high plasma concentrations of the CYP2B6 substrate efavirenz in CYP2B6*6/*6 individuals [38] but are not confirmed by pharmacokinetics of the CYP2B6 substrate bupropion, where CYP2B6*6/*6 individuals did not differ from *1/*1 individuals [39]. The presence of the 516G>T SNP (encoding the Gln172His change; allele frequency 26%), which is present in the *6, *7, *9, and *13 alleles, was correlated with a factor three decreased activity in studies on the CYP2B6 substrate efavirenz [40,41]. In contrast, population kinetic analyses on bupropion hydroxylation studies did not show an effect of the *CYP2B6*5* allele [39]. The conversion of cyclophosphamide to 4OH-cyclophosphamide as a function of total CYP450 protein was not different between *CYP2B6*6/*6* individuals compared to *CYP2B6*1/*1* individuals [31]. The use of CYP2B6 pharmacogenetics for cyclophosphamide or ifosfamide therapy seems to have a certain potential, but awaits proper identification of the relevant SNPs or variant alleles. Interestingly, also a variant allele encoding an increase in CYP2B6 activity was identified: *CYP2B6*4* variant allele carriers (785A>G, Lys262Arg) showed a 1.6-fold higher enzymatic activity measured by bupropion hydroxylation [39].

2.3. *CYP2C8, 2C9, and 2C19*

The CYP2C subfamily, like CYP2D6 responsible for the metabolism of a fifth of clinically used drugs, consists of four members: CYP2C8, CYP2C9, CYP2C18 and CYP2C19; the corresponding genes are clustered on chromosome 10q24. Polymorphisms in the *CYP2C8*, *2C9*, and *2C19* genes have been shown to result in toxicity of specific drugs in affected individuals [42]. With respect to anticancer therapy, CYP2C19 plays a role in the metabolism of cyclophosphamide, ifosfa-

Table 1 – Functional consequence of genetic variation on the activity of anticancer agents

Drug	CYP	Effect	Ref.
Cyclophosphamide	2B6	4-Hydroxylation, activation	[32–35,43,44]
	3A4	4-Hydroxylation, activation	
		N-dechloroethylation, inactivation	
	2C19	4-Hydroxylation, minor contribution	
	2C9	4-Hydroxylation, minor contribution	
	2C8	4-Hydroxylation, minor contribution	
	2A6	4-Hydroxylation, minor contribution	
Docetaxel	3A4	Low-Km	[54]
	3A5	10× Higher Km than 3A4	
	(1A1, 1A2, 2A6, 2B6, 2C8, 2C9, 2D6, 2E1)	minor contribution	
Etoposide	3A4	O-demethylation	[55,56]
	3A5	O-demethylation, <3A4	
	1A2	Minor contribution	
	2E1	Minor contribution	
Gefitinib	3A4	O-demethylation, major pathway	[22,23]
	3A5	O-demethylation, <3A4	
	2D6	Minor pathway (metabolite M523595)	
Ifosfamide	2B6	N-dechloroethylation	[32,34,35,43,44]
		(4-Hydroxylation)	
	3A4	N-dechloroethylation	
		4-Hydroxylation, activation	
	2C9	4-Hydroxylation, minor contribution	
	2C8	4-Hydroxylation, minor contribution	
	2A6	4-Hydroxylation, minor contribution	
	2C19	4-Hydroxylation, minor contribution	
Imatinib	3A4	N-demethylation to active metabolite	[24]
	3A5	N-demethylation to active metabolite	
	2C9	Capable of metabolizing, role unclear	
	2D6	Capable of metabolizing, role unclear	
	2C19	Capable of metabolizing, role unclear	
	1A2	Capable of metabolizing, role unclear	
Paclitaxel	2C8	Major pathway	[57–59]
	3A4	Minor pathway	
Tamoxifen	3A4/5	N-demethylation, inactivation, main pathway	[24,25,27]
	2D6	4-Hydroxylation, activation	
	2B6	4-Hydroxylation, activation	
	2C9	4-Hydroxylation, activation, and N-demethylation	
	2C19	4-Hydroxylation, activation	
Teniposide	3A4	Major enzyme	[55]
	3A5	Minor contribution	
Thalidomide	2C19	5-OH and 5′-OH formation	[45]
Vinblastine	3A	Unidentified metabolites	[60–62]
Vincristine	3A	Unidentified metabolites	
Vindesine	3A	Unidentified metabolites	
Vinorelbine	3A4	Unidentified metabolites	

Abbreviations. CYP, cytochrome P450 isoform; Ref., reference.

mide and thalidomide (Table 1 [43–45]). Three to five percent of Caucasians and 12–23% of Asians are poor metabolizers. 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 [46,47]. Up to now, variant alleles *1–*16 have been described, of which seven (*CYP2C19**2–*8) encode proteins without enzyme activity [16].

CYP2C9 is the principal CYP2C in human liver [48], and variant alleles *2–*20 have been described up to now; from these, the *2, *3, *5, *11, *12, *16 and *18 alleles are indicated to have decreased activity [16]. Two variant alleles *2 (Arg144Cys) and *3 (1075A>C; Ile359Leu) that affect CYP2C9 metabolism in vivo, occur in 11% and 3–16% of whites, and in 3% and 1.3% of blacks, respectively [49].

A threefold lower intrinsic clearance for cyclophosphamide was observed with recombinant CYP2C9.2 and CYP2C9.3 protein when compared to CYP2C9.1 [43,44]. However, no significant differences in cyclophosphamide metabolism in hu-

man microsomes obtained from *CYP2C9*2* or *3 individuals compared to those from *CYP2C9*1* homozygotes could be demonstrated [43]. In general, the role of CYP2C9 in cyclophosphamide activation is thought to be minimal [43].

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. A *CYP2C8*4* (792C>G; Ile264Met) allele with probably decreased activity has been reported [50]. A potential inactive allele (475delA; frameshift: *CYP2C8*5*) was found in one Japanese individual [51]. The clinical consequences of these variant alleles remain to be determined [52]. From the reported variant alleles *1–*10, the *5 and *7 allele are reported to be null alleles while the *8 allele has decreased activity [16]. A linkage disequilibrium has been reported for *CYP2C8* and *CYP2C9*, which means that associations found for CYP2C8 may also be found for CYP2C9, and vice versa. Decreased activity of recombinant CYP2C8.2 and CYP2C8.3 enzymes (the protein products of the *CYP2C8*2* and *CYP2C8*3* gene, respectively) was demonstrated on the metabolism of paclitaxel, for which 85% of the inactivation occurs by conversion to 6OH-paclitaxel by CYP2C8 [53].

2.4. *CYP3A4 and CYP3A5*

CYP3A (cytochrome P450 3A) is the cytochrome P450 isozyme involved in the metabolism of most agents in the treatment of cancer, among which cyclophosphamide, docetaxel, doxorubicin, etoposide, gefitinib, ifosfamide, imatinib, irinotecan, paclitaxel, tamoxifen, teniposide, and vinca-alkaloids (Table 1 [54–62]). Four functional genes, located on chromosome 7q, exist of which *CYP3A4* is the most important one. The first described variant, *CYP3A4*1B* shows large interracial differences in frequency [63], but its relevance for drug metabolism is not yet clear [64–66]. Next to other *CYP3A4* polymorphisms (*2, *3, *4, *5, *6, *17 and *18), relationships with irinotecan metabolism have been explored in Caucasian and Chinese people, but significant results could not be found [67–70]. This is in accordance with a study by Garcia-Martin [71], who found no effect of the *CYP3A4*1B* SNP on protein expression, although other papers do indicate a higher transcription of the *CYP3A4* gene when this SNP is present [72,73]. In general, with the exception of the *CYP3A4*1B* polymorphism, the SNPs described so far for *CYP3A4* are too rare (<1% allele frequency in the Caucasian population [74]) to be of clinical relevance in screening patients prior to therapy.

For CYP3A5, the second most important isozyme of the CYP3A family, the frequently occurring *3 variant allele (92% allele frequency [75]) which encodes a *CYP3A5* splice variant, is responsible for a low expression and activity of CYP3A5 in 80% of Caucasians. Despite this clear effect on enzyme activity, however, no relationship between this variant and irinotecan metabolism could be found, implying that CYP3A5 does not contribute significantly to its metabolism [67–70]. For etoposide, clearance was found to correlate with *CYP3A5* genotype in blacks, but not in whites, leaving the exact role of CYP3A5 in etoposide metabolism unclear [56]. Because of the large substrate overlap between CYP3A4 and CYP3A5, and taking into account 20% active versus 80% inactive CYP3A5 in Caucasians (or 70% active versus 30% inactive CYP3A5 in African Americans), it is to be expected that the clinical importance of the *CYP3A5* SNP with respect to anticancer therapy will become much clearer with time.

3. Phenotyping CYP3A

Apparently, it is still quite hard to determine clear relationships between genetic variation in CYPs and the metabolism of (anticancer) drugs. This is not totally unexpected, as CYPs show a highly variable expression as the result of non-inherited influences. Environmental factors (like food substances), and physiological factors like inflammation, hepatic metastases and other liver diseases are responsible for at least a part of IIV in anticancer agent toxicity and activity. Also co-medication may affect CYP expression, as was seen for the strong CYP3A inhibitor ketoconazole, which led to altered concentrations of chemotherapeutic compounds in patients [76,77]. In addition, for patients co-treated with the popular antidepressants paroxetine and St. John's wort, lowered tamoxifen and irinotecan active metabolite levels, respectively, were noticed [26,27,78]. This was assumed to be due to CYP2D6 inhibition and CYP3A4 induction, respectively [27,78].

As this variable expression in these phase I enzymes exists, genotype alone clearly does not succeed to predict this expression enough. To bypass this problem, "phenotyping" measures have been developed for many CYPs [79,80]. As CYP3A is the most abundantly expressed CYP isotype research has focused on that one, but also for CYP2D6, CYP1A2, and CYP2E1, phenotyping measures have been developed [79]. As "phenotype" is the sum of genetic, physiological, and environmental factors, determination of phenotype is assumed to be a better predictor of a patient's abilities to metabolize certain (anticancer) agents than genotype alone. Drugs which are primarily metabolized by CYPs (like docetaxel) may be first-choice compounds for a CYP-phenotyping test. The phenotyping procedure is straightforward by giving the patient a "probe"-drug, (which is ideally a simple, safe, cheap and easily administered and detectable compound), and which is metabolized by the enzyme in question. With the development of the erythromycin breath test (ERMBT), the first, and currently best studied, CYP3A phenotyping test was invented [81–83]. For other phenotyping tests, the principle is quite comparable. The probe is given (intravenously or orally) and after metabolic transformation in the human body, probe metabolites are collected (i.e., in blood, urine, air), and clearance (among other pharmacokinetic parameters) of the probe can be determined. Next, a CYP3A metabolized anticancer agent is given, and the metabolism of that drug (usually expressed by its clearance) is correlated to the metabolism of the probe. If a clear relationship between the probe and the tested anticancer agents exists, the test may be of predictive value. Up to now, encouraging results have been obtained for docetaxel and probe drugs like cortisol, dexamethasone, erythromycin, and midazolam (Table 2 [82,84–87]). Recently, dexamethasone was also used as a probe for vinorelbine [88]. And for irinotecan, which is partly metabolized by CYP3A, a relationship with midazolam metabolism was seen

Table 2 – Significant correlations between CYP3A probe parameters and the plasma clearance of anticancer drugs

CYP3A probe parameter	Plasma clearance of drug	*n*	*r*	*P*	Ref.
Cortisol					
Ratio 6β-OH cor (urine)/FC	Docetaxel	30	0.867	<0.001	[84]
Dexamethasone					
Plasma clearance[a]	Docetaxel	21	0.51	<0.02	[87]
Plasma clearance[a]	Vinorelbine	20	0.65	<0.01	[88]
6β-OH dex (urine)[a]	Docetaxel	21	0.48	<0.05	[87]
6β-OH dex (urine)[a]	Vinorelbine	20	0.47	<0.05	[88]
Ratio 6β-OH dex (urine)/dex[a]	Docetaxel	21	0.45	<0.05	[87]
Ratio 6β-OH dex (urine)/dex[a]	Vinorelbine	20	0.58	<0.01	[88]
Erythromycin					
% Exhaled $^{14}CO_2$	Docetaxel	21	0.81–0.92[b]	<0.0001	[82]
Midazolam					
Plasma clearance	Docetaxel	31	0.60	0.0005	[85]
Plasma clearance	Irinotecan	30	0.745	<0.001	[69]
t_4	Irinotecan	30	−0.416	0.022	[69]

Abbreviations. CYP3A, cytochrome P450 subtype 3A; *n*, number of patients studied; Ref., reference; 6β-OH cor, 6β-hydroxy-cortisol; FC, free cortisol; 6β-OH dex, 6β-hydroxy-dexamethasone; dex, dexamethasone; $^{14}CO_2$, ^{14}C labeled CO_2; t_4, $t = 4$ h after infusion sample.
a Personal correspondence.
b Triplicate performed test.

as well [69]. Individual probes do not have to correlate with each other and so, the best probe (or probes) for a given drug does not have to be predictive for another drug [69,89,90]. Therefore, relationships have to be determined separately between putative probes and (anticancer) drugs [91]. This requires additional studies to identify clinically applicable correlations for various sorts of (chemotherapeutic) agents.

4. Clinical practice and future perspectives

Although anticancer drug development is ongoing for decades now, next generation cytotoxic agents unfortunately did not show a much improved side-effect profile. Much IIV exists in the seriousness and incidence of toxicities, where neutropenia still remains one of the major dose-limiting ones. Sometimes genetic polymorphisms in enzymes, drug transporters or drug targets may be the underlying reason. Nonetheless, in most cases, relationships with genetic variation are less clear than anticipated. As many enzymes (and drug-transporters) interact, it is often hard to point the clinical effect of one single polymorphism or even a group of linked mutations in a gene. Many non-inheritable factors are involved in the expression of an enzyme too, which also interferes with clinical outcome. Therefore, before hard conclusions can be drawn, studies of genetic variants with putative clinical effects have to be performed prospectively in large(r) cohorts of patients. And, as many polymorphisms are largely variable among different ethnic groups, trials should be done within various ethnic races.

Even if an indisputable relationship between pharmacogenetics, pharmacokinetics and pharmacodynamics for a given drug is found, still many hurdles have to be taken before this knowledge can be used in clinical practice. First of all, advanced techniques to detect these polymorphisms should be applicable in common practice and physicians should be adequately skilled. At this moment, technical developments enable faster and higher throughput genotyping (including real time PCR and DNA chips). The high throughput technology will enable larger studies to be conducted, while the speed at which an individual result will be available is important for incorporation of pharmacogenetics into clinical practice [92]. On the other hand, currently these techniques are not operational in most (general and academic) clinics. Following an Australian questionnaire recently held, only in 2% of all studied facilities in Australia and New Zealand, could genotyping tests be performed [93]. Next, costs should be acceptable. As serious adverse events from drug therapy will lead to higher expenses (i.e., hospitalization, extra pharmacy costs), a prospective pharmacogenetic screening might prevent these negative effects and could partly and indirectly save costs [7]. Also, avoiding expensive treatment failures, and having improved well-being for the patient, should counterbalance the costs made for a genetic test [7,94].

Nonetheless, a certain amount of optimism is justified for the field of pharmacogenetics. It has been said before that pharmacogenomics has the potential to revolutionize cancer therapy [95]. Right now it is hard to tell when this future perspective will become a reality. Also its limitations have to be clear. For instance in the case of irinotecan, pre-treatment *UGT1A1*28* screening may prove to be useful [11,67]. Meanwhile, as CYP3A expression also influences irinotecan metabolism significantly, and genetic variation does not explain the observed variation in CYP3A4 activity [96], phenotyping procedures may have an additional value here. Combining genotyping and phenotyping procedures may be the way to go in the near future for many drugs, in which the emphasis (genotyping versus phenotyping) may depend on the specific drug used.

Conflict of interest statement

None declared.

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